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CONTENTS OF VOLUME XIV

JULY, 1914. NUMBER 1

PAGE

AN ACID-FAST STREPTOTHRIX (NOCARDIA). DAVID JOHN DAVIS, M.D., CHICAGO ... 1

THE BLOOD IN INANITION. J. EARLE ASH, M.D., BOSTON, MASS ... 8

THE PATHOLOGIC PHYSIOLOGY OF THE CIRCULATION DURING HEMORRHAGE. CARL J. WIGGERS, M.D., NEW YORK ... 33

THE BLOOD-PRESSURE IN PNEUMONIA. L. H. NEWBURGH, M.D., AND GEORGE R. MINOT, M.D., BOSTON ... 48

PNEUMOCOCCUS INFECTION AND LOBAR PNEUMONIA. RUFUS COLE, M.D., NEW YORK ... 56

THE COLOR-INDEX OF THE RED BLOOD-CORPUSCLES. ERICH MEYER, M.D., STRASBURG, AND E. E. BUTTERFIELD, M.D., NEW YORK ... 94

HUMAN STREPTOTRICHOSIS AND ITS DIFFERENTIATION FROM TUBERCULOSIS. EDITH J. CLAYPOLE, M.D., BERKELEY, CAL ... 104

A PRELIMINARY REPORT ON THE PRODUCTION, ACTION AND THERAPEUTIC EFFECTS OF LEUKOCYTIC EXTRACTS. R. A. ARCHIBALD, AND GERTRUDE MOORE, OAKLAND, CAL. ... 120

A COMPARISON OF BANG'S MICROMETHOD FOR DETERMINING BLOOD SUGAR WITH BERTRAND'S METHOD. R. FITZ, M.D., BOSTON ... 133

BOOK REVIEWS: DISEASES OF THE STOMACH, INCLUDING DIETETIC AND MEDICINAL TREATMENT. BY GEORGE ROE LOCKWOOD, M.D. ... 143

THE FUNDAMENTAL BASIS OF NUTRITION. BY GRAHAM LUSK ... 143

AUGUST, 1914. NUMBER 2

STUDIES IN THE PATHOLOGY OF THE SPLEEN. JOHN H. KING, M.D., BALTIMORE ... 145

THE INFLUENCE OF THE HIGH-CALORY DIET ON THE RESPIRATORY EXCHANGES IN TYPHOID FEVER. WARREN COLEMAN, M.D., AND EUGENE F. DUBOIS, M.D., NEW YORK ... 168

A STUDY OF TWENTY-NINE CASES OF ADVANCED PULMONARY TUBERCULOSIS TREATED BY ARTIFICIAL PNEUMOTHORAX. LEO KESSEL, M.D., AND MAX TASCHMAN, M.D., NEW YORK ... 210

STUDIES OF ACIDOSIS AND DYSPNEA IN RENAL AND CARDIAC DISEASE. FRANCIS W. PEABODY, M.D., BOSTON ... 236

CLINICAL AND METABOLISM STUDIES IN A CASE OF MYOTONIA CONGENITA — THOMSEN'S DISEASE. JACOB ROSENBLOOM, M.D., PH.D., AND BENSON A. COHOE, M.B., PITTSBURGH ... 263

STUDY OF A CASE OF APLASTIC ANEMIA. JOHN H. MUSSER, JR., M.D., PHILADELPHIA ... 275

CONTENTS OF VOLUME XIV

SEPTEMBER, 1914. NUMBER 3

PAGE

Introduction to the Second Progress Report of the Thompson-McFadden Pellagra Commission. J. F. Siler, M.D., P. E. Garrison, M.D., and W. J. MacNeal, New York 289

A Statistical Study of the Relation of Pellagra to Use of Certain Foods and to Location of Domiciles in Six Selected Industrial Communities. J. F. Siler, M.D., P. E. Garrison, M.D., and W. J. MacNeal, M.D., New York 293

Nitrogen and Sulphur Metabolism in a Cretin. Isidor Greenwald, Ph.D., New York 374

Lymphocyte Increase and Altitude. Minnie E. Staines, M.D., and T. L. James, M.D., Colorado Springs, Colo., and Carolyn Rosenberg, M.D., New York 376

The Glycyl-Tryptophan Reaction in Meningitis. Ralph H. Major, M.D., San Francisco, and Edmund Nobel, M.D., Vienna 383

Elliptical Human Erythrocytes. F. Warner Bishop, M.D., New York 388

The Pituitary Gland in Epileptics. J. F. Munson, M.D., and Arthur L. Shaw, M.D., Sonyea, N. Y. 393

The Specific Rôle of Foods in Relation to the Composition of the Urine. N. R. Blatherwick, New Haven, Conn. 409

Book Reviews: A Practical Treatise on Medical Diagnosis for Students and Physicians. By John H. Musser, M.D., LL.D. 461

Blood-Pressure in Medicine and Surgery. A Guide for Students and Practitioners. Edward H. Goodman, M.D. 462

OCTOBER, 1914. NUMBER 4

The Relation of Methods of Disposal of Sewage to the Spread of Pellagra. J. F. Siler, M.D., Washington, P. E. Garrison, M.D., Washington and W. J. MacNeal, M.D., New York 453

Notes on a Normal, Presystolic Sound. E. W. Bridgman, M.D., Baltimore 475

Periarteritis Nodosa—A Clinical and Pathological Review of the Disease. With a Report of Two Cases. Albert R. Lamb, M.D., New York 481

Latent Hypertrophy of the Heart in the Nephritis of Children. H. Wessler, M.D., New York 517

The Non-Specificity of Carbohydrate Tolerance Tests. Joseph C. Friedman, M.D. and Solomon Strouse, M.D., Chicago 531

The Potassium Content of Cerebrospinal Fluid in Various Diseases. Jacob Rosenbloom, M.D., Ph.D. and Vernon L. Andrews, M.D., Pittsburgh 536

The Relation Between the Phenolsulphonephthalein Excretion in the Urine and the Non-Protein Nitrogen Content of the Blood in Human Cases. Channing Frothingham, Jr., M.D. and Wilson G. Smillie, M.D., Boston 541

A Point to be Considered in the Use of the Abderhalden Reaction. Ellison L. Ross, Ph.D. and H. Douglas Singer, M.D., M.R.C.P., Kankakee, Ill. 552

OCTOBER—Continued

PAGE

THE SPECIFICITY OF CHOLESTERIN WITH SYPHILITIC SERUMS AND OF CHOLESTERIN-REINFORCED HEART ANTIGEN IN THE WASSERMANN REACTION. I. CHANDLER WALKER, M.D., BOSTON 563

BOTULISM: A REPORT OF FOOD-POISONING APPARENTLY DUE TO EATING OF CANNED STRING BEANS, WITH PATHOLOGICAL REPORT OF A FATAL CASE. RAY LYMAN WILBUR, M.D. AND WILLIAM OPHÜLS, M.D., SAN FRANCISCO 589

A NOTE ON THE NORMAL PECULIARITIES OF THE HEART-SOUNDS IN THE REGION OF THE STERNUM. GEORGE BLUMER, M.D., NEW HAVEN, CONN 605

BOOK REVIEW. MODERN MEDICINE. ITS THEORY AND PRACTICE IN ORIGINAL CONTRIBUTIONS BY AMERICAN AND FOREIGN AUTHORS. SIR WILLIAM OSLER, BART., M.D., F.R.S. AND THOMAS MCCRAE, M.D 608

NOVEMBER, 1914. NUMBER 5

THE PULSE-FLOW IN THE BRACHIAL ARTERY. IV. REFLECTIONS OF THE PRIMARY WAVE IN DICROTIC AND MONOCROTIC PULSE-FORMS. ALBION WALTER HEWLETT, M.D., ANN ARBOR, MICH 609

THE TOTAL NON-PROTEIN NITROGEN AND THE UREA OF THE BLOOD IN HEALTH AND IN DISEASE, AS ESTIMATED BY FOLIN'S METHODS. WILDER TILESTON, M.D., AND C. W. COMFORT, JR., M.D., NEW HAVEN CONN. 620

AN EPIDEMIC OF STREPTOCOCCUS SORE THROAT IN JACKSONVILLE, ILL., WHICH WAS TRACED TO THE MILK OF COWS AFFECTED WITH STREPTOCOCCUS MASTITIS. JOSEPH A. CAPPS, M.D., AND DAVID J. DAVIS, M.D., CHICAGO 650

SPECIFIC HYPERLEUKOCYTOSIS. STUDIES IN TYPHOID IMMUNIZATION. IV. FREDERICK P. GAY, M.D., AND EDITH J. CLAYPOLE, M.D., BERKELEY, CAL. 662

AN EXPERIMENTAL STUDY OF METHODS OF PROPHYLACTIC IMMUNIZATION AGAINST TYPHOID FEVER. STUDIES IN TYPHOID IMMUNIZATION. V. FREDERICK P. GAY, M.D., AND EDITH J. CLAYPOLE, M.D., BERKELEY, CAL. 671

A CASE OF DIABETES INSIPIDUS. R. FITZ, M.D., BOSTON 706

CONTRIBUTIONS TO THE EXPERIMENTAL PATHOLOGY OF THE STOMACH. I. EXPERIMENTAL PYLORIC STENOSIS. W. W. HAMBURGER, M.D., AND J. C. FRIEDMAN, M.D., CHICAGO 722

A CLINICAL AND BACTERIOLOGICAL STUDY OF HEXAMETHYLENAMIN AS A URINARY ANTISEPTIC. LOUIS HENRY LEVY, M.S., M.D., AND ABRAHAM STRAUSS, M.D., NEW YORK 730

BLOOD-PLATELETS AND TUBERCULOSIS. GERALD B. WEBB, M.D., GEORGE BURTON GILBERT, M.D., AND LEON C. HAVENS, M.A., COLORADO SPRINGS, COLO. 743

PULMONARY PHYSICAL SIGNS AND ROENTGEN-RAY FINDINGS IN HEALTHY ADULTS. LOUIS HAMMAN, M.D., AND F. H. BAETJER, M.D., BALTIMORE 757

CONTENTS OF VOLUME XIV

DECEMBER, 1914. NUMBER 6

PAGE
Clinical Studies in Caffein. Lester Taylor, M.D., Cleveland 769
Postanesthetic Glycosuria as Influenced by Diet, Body Temperature and Purity of the Ether. E. L. Ross, Ph.D., Chicago, and P. B. Hawk, Ph.D., Philadelphia 779
The Complement-Deviation Test with Besredka's Tuberculin and the Occurrence of Tuberculosis among Syphilitics as Diagnosed by This Test. J. Bronfenbrenner, Ph.D., Pittsburgh 786
A Clinical Study of Two Hepatic Functional Tests (Galactose and Phenoltetrachlorphthalein). Warren R. Sisson, M.D., Boston 804
Study XXII: The Effect of Theobromin Sodium Salicylate in Acute Experimental Nephritis, as Measured by the Excretion of Phenolsulphonephthalein. Henry A. Christian, M.D., Boston 827
Nitrogen Metabolism and the Significance of the Non-Protein Nitrogen of the Blood in Experimental Uranium Nephritis. Herman O. Mosenthal, M.D., Baltimore 844
Family Periodic Paralysis. Report of a Typical Case, with Metabolism Study. Theodore Diller, M.D., and Jacob Rosenbloom, M.D., Ph.D., Pittsburgh 869
Note on the Incidence of Status Lymphaticus in Dementia Praecox. Haven Emerson, M.D., New York 881
Artificial Pneumothorax, with Report of Gas Analyses and Experiments to Determine the Use of Air or Nitrogen. Gerald B. Webb, M.D., G. Burton Gilbert, M.D., T. L. James, M.D., and Leon C. Havens, M.A., Colorado Springs, Colo 883
The Effect of Strychnin, Caffein, Atropin and Camphor on the Respiration and Respiratory Metabolism in Normal Human Subjects. D. L. Edsall, M.D., and J. H. Means, M.D., Boston 897
Some Considerations on the Physiology of the Otic Labyrinth. J. Gordon Wilson, M.D., Chicago, and F. H. Pike, M.D., New York... 911
Index to Volume XIV 921

The Archives of Internal Medicine

Vol. XIV JULY, 1914 No. 1

AN ACID-FAST STREPTOTHRIX (NOCARDIA) *

DAVID JOHN DAVIS, M.D.
CHICAGO

Infections with branching, acid-fast, mycelial organisms of the streptothrix type are sufficiently rare to justify a detailed account of such cases together with a description of the organism. There is perhaps no field in all bacteriology which is more unsatisfactory, so far as classification is concerned, than the study of these higher fungi. The strains appear to be very plastic and hardly any two of them are identical in all details. For many years reports of these infections have been accumulating and at the present time both clinical and bacteriological data are fairly abundant. Much of this information has not been correlated but more recently some important studies have appeared, notably the work of Wright,[1] Musgrave, Clegg and Polk,[2] Foulerton,[3] and especially of Edith Claypole.[4]

Wright has studied exhaustively certain members of this group. He contends that the organisms of the actinomyces type, as found in cattle and in man, are clearly different from other members and maintains that the term, *actinomyces bovis,* first given them by Bollinger should be their proper name. He thinks that the other organisms often classed in this series should be termed after Nocard's work, "nocardia" and the disease "nocardiosis." The nocardia, he says, are "distinguished from the organisms of actinomycosis in that they do not occur in the inflammatory fluids or lesions in man in the compact structures or form the radiate club-bearing structures or granules so characteristic of actinomycoses. Moreover, the organism of actinomycosis does not resist decolorization by dilute acids after having been stained with fuchsin. Localization of the lesions about the mouth and jaw so common in actinomycosis is unknown in nocardiosis." From the point of view of practical medicine there is much to be said

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*From the Department of Pathology, University of Illinois, Chicago.

* Submitted for publication Feb. 5, 1914.

[1] Wright: Jour. Med. Research, 1905, xiii, 349; also Osler's Mod. Med., i, 340.

[2] Musgrave, Clegg and Polk: Philippine Jour. Sc., 1908, iii, 447.

[3] Foulerton: Lancet, London, 1910, i, 551, 626, 771.

[4] Claypole, Edith: Jour. Exper. Med., 1913, xvii, 99.

in favor of Wright's view, for, clinically, infections with actinomyces form a well-defined disease which agrees both in animals and in man in many fundamental particulars.

Claypole has attempted to correlate various strains of this group by immunological tests, especially by the complement-fixation phenomenon. She used a large number of strains isolated from various sources including among others several actinomycetes, *Streptothrix madurae, S. asteroides, S. nocardii, S. eppingeri, S. coprae, Bacillus leprae* and *B. tuberculosis.* Many of the organisms studied show more or less of the acid-fast quality but considerable variation in this respect was noted, the *B. leprae* and *B. tuberculosis* being of course most constant. She concludes that it is impossible to draw a sharp line between the various strains from any point of view, morphological, biological, or serological. They form a more or less continuous series and she indicates the change, which is gradual from the mycelial organisms to the bacillary acid-fast organisms, by curves which represent fixation with any given antigen.

I wish to give here the more important details concerning a case of infection with a typical acid-fast mycelial organism which evidently belongs in the streptothrix group and which, according to Wright, should properly be called a nocardia.

History.—The patient is a native-born American, aged 64, single, a teamster. After being acutely ill for three days he was admitted to St. Luke's Hospital, Chicago, in the service of Drs. Favill and Clark, to whom I am indebted for the clinical data. For several years he has been troubled with a chronic cough and at times raises a large amount of thick tenacious sputum. He has been working around horses, but none of the animals so far as he knew had been ill. He has not decreased in weight for several years past. One morning the patient was awakened by a severe, sharp stabbing pain in the right chest. The pain was constantly present but was increased by deep breathing or movements of the body and arms. The patient also had several severe chills later in the day. The pain gradually increased on the following day and severe dyspnea developed, making it impossible for him to lie down. The dyspnea was very severe for a few days and then became less. On the day following the acute onset he began to have a loose cough with expectoration of much white, tenacious, odorless sputum. Excepting the respiratory system no findings of any significance were noted. The following data were obtained at the time he entered the hospital and when he was acutely ill.

Examinations.—There is some hyperemia of the pharynx and uvula and some greenish mucus is adherent to the pharyngeal wall. The chest is well developed; there is a marked depression over the supra- and infraclavicular fossae. Dyspnea is marked and the expansion is deficient, being very much restricted on the right side. The respiration is 28 per minute and is very shallow. There is a frequent difficult cough with expectoration of abundant greenish tenacious sputum. The same conditions of expansion are revealed by palpation as by inspection. Tactile fremitus is much greater over the right chest anteriorly and posteriorly above the level of the angle of the scapula. Below that line on the right side posterior to the axillary line, it is practically absent. The breath sounds are normal over the left lung. On the right posterior side there is an area of flatness over which the sounds are absent or very distant. Over the area of relative dulness, posteriorly, the sounds are weaker than over the corresponding area of the opposite side and coarse bubbling râles on inspiration are heard over the right lower lobe anteriorly and posteriorly. There is marked pyorrhea. The spleen is not enlarged and the outlines of the stomach and liver are normal. The cervical glands are just palpable but not tender. Other glands are not enlarged.

Two roentgenologic examinations made soon after the patient's admittance showed increased density over one-third of the right lung, chiefly in its lower portion. The plates present the appearance of tuberculosis of the lungs.

The patient's respirations during a period of about ten days ranged between 28 and 44 per minute. Later the number of respirations gradually diminished to about 20 to 25 and then remained constant. The pulse was irregular, ranging from 80 to 130.

The temperature chart of this patient is interesting (Fig. 1.) With remarkable regularity the temperature fluctuates from normal or thereabout in the morning to 101 or 102 in the afternoon. After the first ten days the temperature became decidedly lower; but a few days later the fever was again more marked. After leaving the hospital regular temperatures were not taken, but occasional temperatures indicated a gradual decline and at present, four months after the acute onset, there is no fever.

The leukocytes on admission were 14,500 and four days later were 15,000. Differential count gave polynuclears 86 per cent., small lymphocytes 8 per cent., and large lymphocytes 5 per cent. The results of blood-culture and urine examinations were negative.

The patient left the hospital after about four weeks. Examination at that time showed that the tactile fremitus had returned below the level of the angle of the scapula on the right side. More breath sounds were heard over the area where they were formerly very weak. There were a few coarse dry râles at the end of inspiration over the lower right lobe. Percussion revealed the same dulness except that it was not so pronounced.

On admission to the hospital tubercle bacilli were searched for in the sputum without result, but during the search, an acid-fast, Gram-positive, branching, mycelial growth was found in the sputum. In twelve different sputa examined during the next two weeks these organisms were constantly found. Many pneumococci, a few streptococci and many bacilli, both Gram-positive and Gram-negative, were also present. The cocci were especially numerous during the acute attack but some were always seen, together with the large number of bacilli, after the acute symptoms had subsided.

Since the patient left the hospital he has been under observation and, from time to time, the sputum is examined. He has improved decidedly. He has had no fever or acute symptoms since, but a slight cough still continues and he raises a small amount of mucoid, slightly purulent sputum. Before the patient left the hospital potassium iodid in moderate doses was given for a time and then discontinued. The organisms were not found in the sputum subsequent to this on several examinations, but about three weeks later they reappeared in small numbers. At present they cannot be found in the sputum.

Whether or not the potassium iodid had anything to do with their disappearance is difficult to say, but such a view is suggestive in view of the fact that others[5] have noted good results in these cases following potassium iodid and also in view of the well-known virtues of potassium iodid in other more or less closely related infections, such as sporotrichosis and actinomycosis.

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[5] Kremers, E. D.: U. S. Army (personal letter).

The streptothrix was very numerous in some specimens of sputum, there being several clumps in nearly every field. In other specimens again, they were difficult to find. In the thick greenish tenacious masses of sputum they were more abundant than in the more mucoid portions. Careful search in all specimens failed to reveal masses that in any way resembled the granules or "sulphur bodies" of actinomycosis. There was never any appreciable odor to the sputum.

Microscopically the organisms appear in small clumps of tangled mycelium with distinct branches (Fig. 2). The filaments are irregular and often at the tip is a bulb-like expansion. No definite ray formations with clubs were ever seen. The mycelial filaments are rather fine but are slightly thicker than a tubercle bacillus. They are Gram-positive throughout. They stain readily with carbolfuchsin and resist decolorizing with acid-alcohol. This resistance is distinctly less than that of tubercle bacilli. The following data were obtained with reference to the alcohol and acid-resisting properties of this particular strain in the sputum. Ninety-five per cent. alcohol for three minutes does not decolorize them nor does 2 per cent. nitric acid in 95 per cent. alcohol for three minutes. In one minute 10 per cent. nitric acid alcohol does not decolorize but after three minutes they are completely or nearly completely decolorized. Thirty per cent. acid alcohol destains completely in one minute. Aqueous acid solutions decolorize approximately like the alcohol acid mixture.

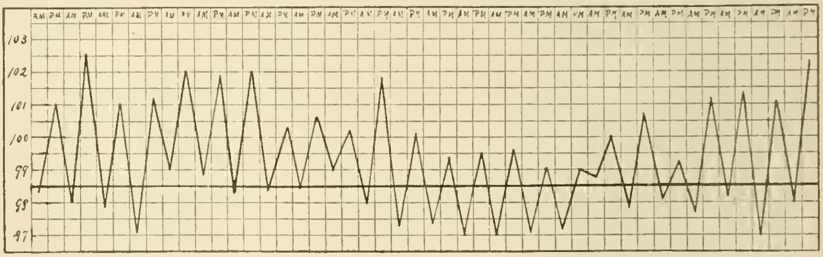

Fig. 1.—Temperature chart of author's patient.

A point of interest is their failure to resist antiformin. Mixtures of these organisms and tubercle bacilli treated with antiformin clearly showed the presence of the tubercle bacilli and the complete disappearance of the streptothrix. In this respect they resemble the actinomyces types more closely than the tubercle bacillus.

Numerous attempts were made to cultivate these organisms. Sputum containing large numbers in smears were streaked on various mediums, including sugar, blood and ascitic agar. Also plate-cultures and deep tube-cultures were made in various mediums. The cultures

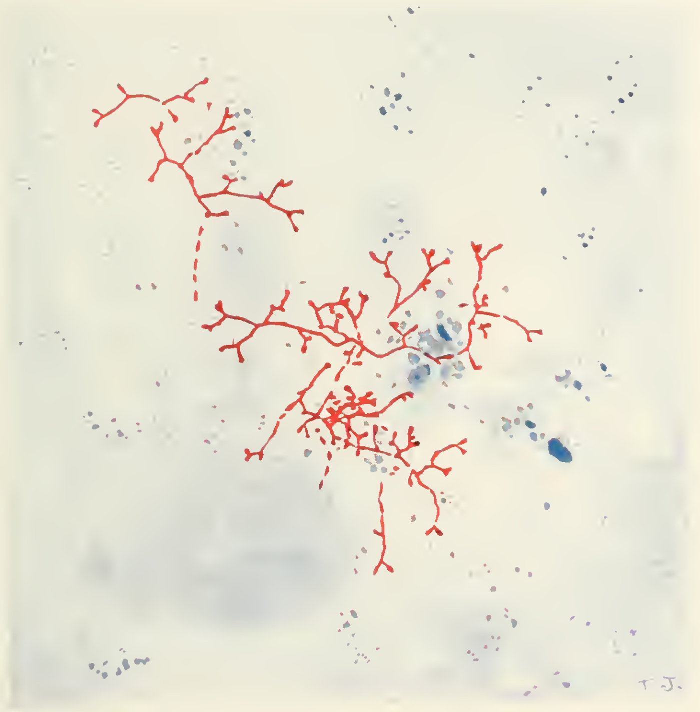

Fig. 2.—Acid-fast streptothrix (Nocordia) appearing in small clumps of tangled mycelium with distinct branches, frequently with a bulb-like expansion at the tips.

Illustrating Article by David John Davis, M.D.
The Archives of Internal Medicine

were carefully observed for weeks and in some instances months for the possible development of the mycelial growths. In no instance was there any growth or multiplication of the streptothrix either under aerobic or anaerobic conditions or at room or incubator temperatures. In this connection mention should be made of the fact that always in the sputum myriads of other germs were present which could have interfered with the development of the streptothrix. There has not been as yet in this case any opportunity to obtain fluids, such as pleural fluid, which might contain the organisms pure. During the acute stage there was some evidence of fluid in the pleural cavity and a few clear drops were obtained by puncture. Unfortunately, this fluid was not preserved for cultures. It is unlikely, however, that any organisms were present, inasmuch as there was no further accumulation later nor were there signs at any time of empyema.

Six guinea-pigs, four young rabbits and a number of white rats were inoculated both subcutaneously and intraperitoneally with moderate and large doses of emulsions of the sputum, which by smear was shown to contain many of the acid-fast organisms. Usually a small induration or an abscess formed at the site of injection, and examination at varying intervals later revealed many bacteria. In no instance did smears stained by the Gram method and by carbolfuchsin show the presence of organisms of the streptothrix type. The lesions all healed after the course of from one to two weeks and in none of the animals did lesions develop that were at all suggestive of this type of infection. It is to be noted that these animal experiments exclude the possibility of these acid-fast organisms being forms of tubercle bacilli.

In the literature on this subject it seems that, while the terms "streptothrix," "cladothrix," "nocardia," "oospora," and "actinomyces" have all been applied to these organisms, the generic term "streptothrix" is being used for this group by most pathologists. J. H. Wright and a few others insist, as pointed out above, that "nocardia" is their proper name because it conforms to the rules of botanical nomenclature. Without going into a close analysis of this matter (which has already been done by several writers), I shall merely say that it seems convenient to use the term "streptothrix" as a generic term, and that it is also convenient to look on these weakly acid-fast mycelial organisms as a subgroup which lies between the organisms of the actinomyces type which are non-acid-fast on the one hand and the strongly acid-fast organisms of the tuberculosis group on the other.

Considerable variation exists among these acid-fast organisms. The one described by Nocard[6] in 1888, as the cause of a disease of cattle known as *farcin du boeuf,* is acid-proof and is distinctly pathogenic for guinea-pigs, producing lesions of a tuberculous character. Eppinger[7] was the first to isolate and describe in a human infection an acid-fast streptothrix which he called *Cladothrix asteroides* and which is now usually referred to as *Streptothrix eppingeri*. This case presented pulmonary lesions and a cerebral abscess. Since then about thirty cases have been described by various writers and in various countries. In a number of cases the organisms were not isolated in culture nor were animal experiments successful. In these respects the various strains seem to vary widely from each other. Some of the organisms grow quite readily on artificial culture mediums, as for example *Streptothrix eppingeri*. Others grow with very great difficulty or not at all, as was true of the organisms from the patient here presented, and also of the organisms from Flexner's case,[8] from that of Buchholz,[9] and also from the case recently reported by Steele and Lee.[10] In certain cases in which, from the symptoms and post-mortem examination or from the smear preparations, there can be no reasonable doubt that they were of this type, no attempt was made to cultivate the organisms nor were animal experiments made. This, for example, is true of the report of Butterfield[11] in which case at necropsy was found a well-defined lung lesion in the walls of which were many acid-fast mycelial organisms.

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[6] Nocard: Ann. de l'Inst. Pasteur, 1888, ii, 293.

It is a point of interest that nearly all these infections involve the respiratory tract, where they produce lesions which can not be differentiated clinically from pulmonary tuberculosis. In some of them, as was true in this case, acute symptoms with signs of bronchopneumonia or abscess may supervene. Empyema is also a common complication and secondary infections, as might be expected, practically always complicate the course of the disease.

Another point that should be mentioned is the possibility that a small acid-fast fragment or filament might be mistaken for a tubercle bacillus. In the sputum of the preceding case, here and there, could be seen small red filaments which, were they seen alone, might be misinterpreted. In order to differentiate them from the tubercle bacilli the following points should be noted. The filaments are slightly thicker than tubercle bacilli. They are decolorized more readily and by weaker acids than tubercle bacilli. They do not resist antiformin. Furthermore, negative results with guinea-pig inoculations will practically exclude tuberculosis.

Sometimes tubercle bacilli show a considerable degree of branching and it is possible that their identity can only be demonstrated by guinea-pig inoculations. This has been shown by W. H. Smith of

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[7] Eppinger: Beitr. z. path. Anat. u. z. allg. Path., ix. 276.

[8] Flexner: Jour. Exper. Med., 1898, iii. 435.

[9] Buchholz: Ztschr. f. Hyg. u. Infectionskrankh., 1897, xxiv. 470.

[10] Steele and Lee: Boston Med. and Surg. Jour., 1913, clxix. 502.

[11] Butterfield: Jour. Infect. Dis., 1905, ii. 421.

Boston, who reports three cases in which branching, acid-fast bacilli were found and which, in animals, caused typical tuberculosis. Attention, however, should be called to the fact that some of the streptothrix strains may produce in guinea-pigs lesions simulating tuberculosis, so that in such cases one should resort to other confirmatory tests. The differentiation of these organisms from actinomyces has already been stated in the first part of the paper.

The question may very properly be considered whether the acid-fast organism here observed was pathogenic or simply a contaminating saprophyte. With the data at hand the question can perhaps not be definitely decided but I believe it is reasonable to assume that this organism is concerned in some way in the production of the pulmonary lesions observed in this case. It is true that acid-fast organisms of the streptothrix type have been seen in sputum in which the observers considered it a saprophyte; but this is very rare. In nearly all cases in which such organisms have been observed in sputum there is strong evidence that they were concerned in a pathologic process. It is interesting in our case to note that it was in the thick, greenish, highly purulent masses of sputum that the mycelial growths occurred, whereas in the less purulent and more mucoid parts of the sputum they were rare or not present at all. Saprophytic organisms, as a rule, grow fairly readily, whereas these organisms, under the varied conditions of cultivation attempted, did not grow at all. It should be said further that the results of animal inoculation with organisms of this group are not reliable so far as determining the pathogenicity for human beings is concerned. This is well exemplified by the organisms of the actinomyces type which have been far better studied in this respect than the acid-fast mycelial organisms. With them the result of animal inoculations in the lower animals is almost invariably negative in spite of the injection of large doses of organisms recently isolated from active lesions. While positive results have been obtained with certain strains of acid-fast organisms by inoculation of animals, especially guinea-pigs, it is undoubtedly true that many other organisms of this type are, in this respect, similar to the organisms of the actinomyces type.

SUMMARY

There is a fairly well-defined group of infections, the great majority of which are pulmonary, caused by weakly acid-fast branching mycelial growths (nocardia) which do not form ray-like granules, These cases clinically are not ordinarily distinguished from pulmonary tuberculosis. A case is cited above in which the pulmonary lesions were very probably caused by such an organism. For the treatment of such cases potassium iodid should be given in large doses.

THE BLOOD IN INANITION *

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Under the supervision of Dr. Francis G. Benedict, director of the Carnegie Nutrition Laboratories in Boston, Mr. Levanzin of Malta fasted for thirty-one days. The full report of the extensive observations made on him during this experience will appear shortly as a Carnegie publication. I am deeply indebted to Dr. Benedict for his permission to make this preliminary report on the blood which I examined. The report includes a somewhat curtailed analysis of the literature and the bibliography is limited to the references quoted. The details of technic and the correlations with the other findings are omitted.

The blood has frequently been studied during inanition, but an exhaustive search through the literature brought to light only a few records of systematic examinations covering so long a period of fasting in man as the case that forms the basis of this report. More or less authenic accounts of long fasts are rather numerous, beginning with those of the early religious rites as recorded in the Bible and continuing to the present day. They have been undertaken from diverse motives, religious, scientific, suicidal, personal aggrandizement, as a fad, and without motive, the result of diseased body or mind.

Richet[1] gives references to some long fasts in the eighteenth century and earlier. More specific are such cases as the one reported by Maples[2] of the woman evidently catatonic, who died after abstaining from food for fifty-five days; Frazier's[3] patient, who lived fifty-four days without nourishment; the German, reported by Voelkel,[4] who chose starvation as a means of suicide and accomplished his end after twenty-four days; "the fasting woman of Aurangabad" (Brown[5]), who was able by her cunning to give the impression, at least, of having

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[1] Richet, M. C.: L'inanition chez l'homme, Rev. sc., Par. 1889, series 3, xliv, 106.

[2] Maples, J. H.: A Fast of Fifty-five Days, Med. Brief, St. Louis, 1904, xxxii, 905.

[3] Frazier, B. C.: Prolonged Starvation, Louisville Month. Jour. Med. and Surg., 1908-9, xv, 147.

[4] Voelkel, A.: Freiwilliger Hungertod, Deutsch. med. Wchnschr., 1886, xii, 533.

[5] Brown, E. H.: The Fasting Woman of Aurangabad, Med. Rec., Calcutta, 1890, i, 102.

gone without food and drink for years; Merlatti's[6] public fast of fifty days; also the public and well-controlled fast of Dr. Tanner, which lasted for forty days, being absolute for the first fifteen, the report published by Wilder[7]; and A. Jacques' two fasts, the first a private one of forty days and the other a public exhibition lasting thirty days, both reported by Noël Paton and Stockman.[8] These cases, however, are of interest only in establishing some idea as to how long man can exist on his own tissues, as practically no observations were recorded on them, except some chemical data in the instance last referred to. Fasts of man conducted under scientific supervision and including blood examinations are limited in number, though the work on animals has been rather prolific.

Among the earliest references are those to the work of Valentin[9] in 1838, who concluded that there was no alteration in the relation of blood weight to the body weight as a result of exhaustive starvation and that of Bidder and Schmidt[10] in 1852, though the latter do not report anything more specific than an increase in the solid constituents in the blood of a starving cat. With reference still to the blood as a whole, London,[11] much later, studying a series of eight rabbits from which both food and drink were withheld, found a loss in total quantity, proportional, though, to loss of body weight. Paschutin[12] concludes as the result of the wofk of Heidenhain, Panum and Voit, that the blood is not impoverished by fasting; on the contrary, in certain periods the organism is plethoric. He holds it as remarkable that the number of erythrocytes increases, probably, however, only because of the rapid decrease in plasma. The latter fact is demonstrated markedly in the dogs observed by W. Müller and Buntzen[13] though in none of their animals did a loss of more than 15 per cent. in body weight occur. Luciani[14] holds that aside from water content, the blood exhibits a resistance similar to the nervous system and that the apparent fluctuations, in corpuscular content at least, depend chiefly on the amount of water consumed. Chossat (quoted by Paschutin[12]), however, considered that, next to the fat, the blood suffered the greatest loss, amounting even to 75 per cent. of its former weight. This view is not tenable in the light of practically all other work and was evidently the result of faulty observations. Paschutin[12] quotes Valentin as noting striking general changes during hibernation, in part, that the blood putrefies from two to four times more slowly, that the arterial blood is not so bright a red, and that the venous blood is not so dark as normally, because of disturbance of oxygen interchange.

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[6] Merlatti, Stefano: Histoire d'un jeûne célébre, etc., Paris, 1887.

[7] Wilder, A.: Dr. Tanner and His Famous Fast, Am. Med. Jour., St. Louis, 1896, xxiv, 529.

[8] Noël Paton, D., and Stockman, R.: Observations on the Metabolism of Man During Starvation, Proc. Roy. Soc., Edinburgh (1888-9) 1890, xvi, 121.

[9] Valentin: Repel. f. Anat. u. Physiol., 1838, iii, 156.

[10] Bidder and Schmidt: Die Verdauungsfahrte in der Stoffwechsel, 1852, p. 328.

[11] London, E. S.: Note sur la question du changement de la quantité générale et de l'alcalinité du sang dans le jeûne absolu., Arch. des. sc. biol., 1895-96, iv, 516 (abstract by Mühlmann[17]).

[12] Paschutin: Pathological Physiology, Inanition, 1902, vol. ii, pt. i, 81 (Russian).

[13] Müller, W., and Buntzen: Transfusion and Plethora, Christiania, 1875.

[14] Luciani, L.: Das Hungern, Leopold Voss, Hamburg u. Leipzig, 1890.

Considering more specifically the blood elements and beginning with the erythrocytes, we find that as early as 1843 Schultz[15] and in 1856 Jones[16] observed atrophic and decomposed cells in starving animals. Others since then have noted these striking alterations in shape and size of the corpuscles, among them Manasseïn,[17] Andral-Gavarret,[17] Laptschinski,[17] Tanner[17] and especially Kagen,[18] who studied dogs and rabbits. He found little change in the first days, but as the fast progressed the red cells became smaller and crenated ones appeared more frequently until at the end, many "star forms" were seen and microcytes predominated. Liuboumdrow[19] also found variations in the character of the red cells, macrocytes, microcytes and nucleated cells being common, especially the large form which reached 20 per cent. to 30 per cent. of the total red count. Curtis[20] made systematic observations of Griscom's blood during his forty-five-day fast in 1880. This constitutes, by the way, the longest period with examinations of which any record could be found. Curtis describes in great detail marked alterations in shape, size and consistency of the red cells, illustrating not only the painstaking care with which the observations were made, but somewhat the comparative crudity of the methods employed at that time. As will be seen it is only in the earlier reports that the appearance of macrocytes, microcytes, crenated and distorted cells are recorded and it has occurred to the writer that with the improvement of blood technic, the occasion for their presence was eliminated.

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[15] Schultz: Beitr. z. phys. u. path. Chemie. von Simon, 1884. (Quoted by Mühlmann[17]).

[16] Jones: Smithsonian Contributions to Knowledge, 1856.

[17] Mühlmann, M.: Russische Literatur über die Pathologie des Hungerns, Centralbl. f. allg. Path., 1899, x, 160.

[18] Kagen, J.: Blood and Blood-Pressure in Starving Organisms, Diss. St. Petersburg, 1884. Russian; from the Laboratory for General and Experimental Pathology, Prof. W. Paschutin, St. Petersburg.

[19] Liuboumdrow, P.: Changes in the Blood and Organs in Starvation, Diss., 71, 1893, Russian; from the Laboratory of path. Anat., Prof. W. Winogradow, St. Petersburg.

[20] Curtis, Lester: Physiology of Autonutrition; 1. Study of Blood During a Prolonged Fast, Am. Assn. Advanmt. of Science, 1880, xxx, 95.

In 1887 Senator[21] reported finding a number of microcytes on the thirteenth day of a *Schlafsucht* in which a woman fifty-four years old lay for about seven weeks, receiving as nourishment only a small amount of milk and wine. In Succi's[22] blood, late in the forty-day fast made as a public exhibition in London in 1880, numbers of imperfect blood disks were observed. Charteris,[23] on the other hand, could find no alteration in shape, size or staining qualities of the erythrocytes in the blood of his human subject during the fast of fourteen days in 1907. A few nucleated ones did appear during the last four days. Two examinations were made on Dr. Gayer's blood by Dr. Wile.[24] On the eighteenth day the red cells numbered 5,192,000 and on the thirtieth, 5,776,000, a slight rise. The absence of anisocytosis or any degeneration of cells is mentioned specifically in the report.

In none of the other reports are the characteristics of the individual red cell noted, so it is most likely that no alterations of importance occurred.

Continuing the consideration of the effects of inanition on the numerical estimation of the erythrocytes, the results are found to be at rather wide variance.

Senator[21] found practically no change during the long period of almost complete inanition mentioned above. He attributed little value to the small number of counts he made, but concluded there was probably a slight diminution. During both Cetti's eleven-day and Breithaupt's six-day fasts studied by Senator, Lehman and others[25] an increase was noted, amounting, in the former subject to a million. Dupérié[26] also claims that a considerable increase in number occurs. From the study of Succi's blood during a thirty-day fast supervised by Luciani,[14] the latter concludes that the variations noted in numbers are only relative to the concentration or dilution of blood, from differences in water content. Malassez[17] and Lépine[17] found that while in the beginnings of the fasting periods there was an increase in number, in the later days a decrease occurred.

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21. Senator, H.: Ueber einen Fall von sog. Schlafsucht mit Inanition, Charité-Annalen, 1887, xii, 316.

22. The Fasting Man, Brit. Med. Jour., 1890, i, 1444.

23. Charteris, Francis J.: Record of Changes Observed in the Blood Count and in the Opsonic Power of a Man Undergoing a Prolonged Fast, Lancet, London, 1907, ii, 685.

24. Dr. Gayer's Fast, a personal communication from Dr. Wile of New York City.

25. Senator, Lehman et al.: Untersuchungen über zwei Hungernden Menschen, Virchow's Arch. f. path. Anat. u. Physiol., 1893, cxxxi, Supplementheft, p. i.

26. Dupérié: Sur les variations physiologiques dans l'état normal des globules du sang, Paris, 1878; cited by Rollett in Hermann's Handbuch der Physiologie, iv, part 1.

Clinical records furnish us with the two following reports. The first is one of Landouzy's patients studied by Malassez.[27] A boy of eighteen lived three months and twenty days with a stricture of the esophagus, the result of swallowing sulphuric acid. He obtained practically no nourishment as he vomited food administered by tube. There was a striking loss, the red cells numbering 3,200,000 the day he died. The second case, reported by Brouardel[28] was a man of forty-eight, who lived four months and twelve days after an experience similar to that of the boy mentioned above. But one blood examination was made, and that two days before death, when the erythrocytes were 4,849,000 and the leukocytes 7,852. While I would regard these as practically normal counts, the author of the report concludes that they demonstrate a concentration of the blood.

Kagen,[18] in 1883, claimed that the ordinary methods of determining the cell content of the blood were open to so many sources of error that the results were not dependable. He limited his observations, therefore, to the direct estimation of the solid constituents, the specific gravity (by pyknometer) and hemoglobin content (Malassez's hemochronometer). He examined six dogs and found in the early days an increase in all three factors, attributing the result to concentration through water loss. The amount of solid constituents, he maintains, can equal even at the end of the fast that present under normal conditions.

Liuboumdrow[19] noted as an average of observations on seventeen dogs a slight increase in erythrocytes until the loss of body weight amounted to from 10 to 15 per cent., then a steady decrease until death, the diminution amounting to as high as 32 per cent. on the twenty-eighth day. Nasse[29] also found an increase in number in a dog after eleven days of complete fasting. As proof that this was due to variation in water content, he states that he obtained a reaction in the opposite direction when the animal was again allowed water.

Poletaëw[30] studied eight dogs that received neither food nor water, dying after loss of 50 per cent. in body weight. These all showed an increase in red cells until late in the fasts, after a loss of 30 per cent. body weight, when there was a gradual decrease till death. Poletaëw is not satisfied with the explanation of this finding simply on the grounds of concentration, for he found an increase also in the dogs

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27. Malassez: Bull. et mém. Soc. méd. d. hôp. de Paris, 1874, xi, p. 124.

28. Brouardel: Union Méd., 1876, series 3, xxii, p. 408.

29. De Martigny and Nasse: Ueber den Einfluss der Nahrung auf das Blut, Marburg and Leipsic, 1850.

30. Poletaëw, P.: The Morphologic Composition of the Blood in Complete and Incomplete Starvation in Dogs, Diss. 97, 1894, St. Petersburg (Russian); from the Laboratory of Pathological Anatomy, Professor Uskow.

that were allowed water. He holds that while there may be interference with blood formation, there is also less destruction for bile formation.

Tauszk[31] in a study of Succi's blood during one of his thirty-day fasts, found, after a short interval of decrease, a moderate increase in the red cells. Daiber[32] in 1896 drew his conclusions as to the effect of inanition on the blood, from his findings in Succi's urine during a twenty-day fast. There was an increase in urobilin and earthy alkaline phosphates, both of which to be accounted for by assuming an enormous destruction of erythrocytes, though he does concede that the phosphates might have come from tissue destruction elsewhere. As proof of the adaptability of the blood to altered conditions, he presents the decrease in urobilin and disappearance of phosphate sediment noted after the fifth day, which shows an acquired resistance to the previously destructive influence of fasting. The urobilin was distinctly demonstrable throughout the fast, though greatly reduced, but the phosphates sediment was replaced by urates. The plasma remained intact, as no transudation of its constituents, particularly albumin, through the kidneys could be demonstrated. Daiber concludes that the conditions during inanition must resemble those present in continued fevers in which there is usually red-cell destruction sufficient to give rise to a demonstrable anemia. In these cases, urobilin is present in the urine in distinctive amounts.

Hayem[33] and Reyne[34] each found an increase in erythrocytes in the dog they studied, both animals dying on the twenty-fifth day of fast. Charteris,[23] however, in the case of his already mentioned, could find no suggestive variation, though there was some daily fluctuation.

Gordon,[35] studying the blood of Martin, a medical student who underwent a nine-day fast, could find practically no variation in red-cell count, except that on the sixth day of refeeding, it was about 1,000,000 below the normal. At the end of the first week of Succi's fourth fast, one of forty days conducted in London,[22] the red cells numbered 6,500,000, an increase of 1,000,000 over the average normal individual's count. It may be more or less in this particular case, as the normal count is not given. A. R. Diefendorf, however, found a slight diminution during and a relatively rapid rise immediately following the two fasts of seven and four days, interrupted by a feeding period of nineteen days, which formed the basis of Benedict's[36] report.

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[31] Tauszk: Jahresb. ü. d. Fortschr. d. Thier-Chem., 1894, xxiv, 147; abstr. from Orvosi hetil., Budapest, 1894, p. 512.

[32] Daiber: Beitrag zur Kenntnis des Stoffwechsels beim Hungern, Schweitzer Wchnschr. f. Chem. u. Pharm., 1896, No. 42, xxxiv, 395.

[33] Hayem, G.: Leçons sur les modifications du sang, Paris, 1882, p. 382.

[34] Reyne: Quoted by E. Bardier in his article on Inanition in Dictionaire de Physiologie. Charles Richet, ix, 99.

[35] Gordon, Martin A. A.: A Prolonged Fast, Montreal Med. Jour., 1907, xxxvi, 482.

Three counts were made on the blood of Dr. Penny,[37] who fasted for thirty days, in 1909, drinking only distilled water. They demonstrated a moderate increase till the twentieth day and a loss of 1,000,000 during the remaining ten days. Here again no normal count was obtained. Ronsse and van Wilder[38] hold that there will always be a slow increase in erythrocytes if water as well as food is withheld.

Though the conditions are not altogether analogous, it is interesting to note that in hibernating animals there is a decided decrease in erythrocytes, as is reported by Ranke.[39]

HEMOGLOBIN

Senator, using the von Fleischl method, noted a moderate increase in his woman subject[21] and in Breithaupt;[25] a loss, however, of about 20 per cent. in Cetti[25] in nine days; Liuboumdrow[19] (with Malassez's method) found a slight increase in the blood of dogs until a loss of from 10 to 15 per cent. of body weight had occurred, when a decrease was recorded, that progressed until the animals died; Benedict[36] reports a slight loss during the fasts, with a rise afterward, corresponding to the fluctuations of the erythrocytes. The von Fleischl and Tallqvist methods were used. Martin's[35] hemoglobin was 90 per cent. the first day, 95 per cent. the fourth and 90 per cent. again on the last day, the 9th, dropping to 80 per cent., six days after resuming food. Penny[37] showed an increase of 8 per cent. during his thirty-day fast, going from 104 to 112 per cent. Luciani[14] concludes that there is an actual loss of hemoglobin. Quoting his studies with Bufalini carried out in Sienna in 1882 on a dog that lived fifty-three days without food, he states there was a rapid rise during the first six days (modified Bizzozero's method used). This he explains as being due not only to concentration from loss of water but also to the more rapid consumption of plasma than corpuscles.

Gayer's[24] percentage rose from 80 on the eighteenth day to 100 on the thirtieth, when his fast was broken. Charteris[23] reporting the fourteen-day fast, notes a drop from 110 per cent. to 96 per cent. after remaining unaffected for the first few days. The loss was not recovered until several days after breaking fast. Subbotin[40] using Preyer's method (spectroscope) found a decrease of hemoglobin in a patient fed on nitrogen-free diet. By the 26th day it had fallen from 13.80 per cent. to 11.65 per cent. and on the thirty-eighth day it was 9.52 per cent. In starving rabbits, however, there was an increase, due, he concludes, to decrease in water content of the blood. In a dog that starved for thirty-eight days, there was very little variation, the range being from 13.80 at the beginning to 13.33 per cent. at the end of the fast.

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[36] 36. Benedict, Francis G.: The Influence of Inanition on Metabolism, Carnegie Publication, No. 77, 1907.

[37] 37. Penny, F.: Notes on a Thirty-Day Fast, Brit. Med. Jour., 1909, i, 1414.

[38] 38. Ronsse, I., and van Wilder, H.: Variations du nombre des globules rouges et du taux de l'hémoglobine au cours de l'inanition chez le lapin, Arch. internat. de pharm. et thér., 1903, xl, 301.

[39] 39. Ranke, Johannes: Grundzüge der Physiologie des Menschen, Ed. 3, p. 380.

Hermann[42] in discussing the subject says that the changes found with the ordinary methods of examination may well be due to concentration, because the water is the most variable of the blood constituents. Results are conclusive, therefore, only when they deal less with the hemoglobin content than with the relation of hemoglobin to the total quantity of solid constituents. Groll's[43] work was carried out on this line in rabbits, cats and one dog. The simple hemoglobin percentage showed a rise in all the animals, being, however, only a relative increase, due to concentration of the blood, while there was no actual diminution in the total solids. He concludes that the hemoglobin is more stabile in starving conditions than any of the other constituents.

It would seem from the preceding data that the red cells and hemoglobin are particularly resistant, though in the long fasts there is no doubt a slight loss. The general consensus of opinion appears to be that concentration of the blood through water loss is responsible for the increase found.

LEUKOCYTES

More attention has been paid the white cells than any of the other blood constituents and reports are more at variance as to just what does happen to them during states of inanition. Almost every possible change, especially numerical, has been observed at one time or another. Morphologic alterations are recorded by Luciani.[14] He noted a decrease in size that appeared early, so that by the fifth day all the leukocytes were smaller than the red cells. They recovered their normal size by the ninth day, however. Charteris,[23] on the other hand, mentions specifically that he observed no alteration in size. Manasseïn[17] reports the presence in the leukocytes of fasting rabbits of refractile bodies that are not affected by acetic acid. Källmark[44] observed in his rabbits rarifications in the basophils with agglutination and peripheral arrangement of the granules.

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[40] Subbotin: Ztschr. f. Biol., vii, 187.

[42] Hermann, L.: Untersuchungen über den Haemoglobin-Gehalt des Blutes bei vollständiger Inanition, Arch. f. d. ges. Physiol. (Pflüger's), 1888, xliii, 239.

[43] Groll, S.: Untersuchungen über den Haemoglobin-Gehalt des Blutes bei vollständiger Inanition; Diss. 1887, Königsberg i. Pr.

[44] Källmark, F.: Zur Kenntnis des Verhaltens der weissen Blutkörperchen bei Inanition, Folia Haemat., 1911, xi, pt. i, 411.

Curtis[20] observed peculiar bodies, resembling leukocytes but larger, consisting of spherules too small to measure. These cells measured 1/2000 inch in diameter and exhibited ameboid movement. When these very indefinite bodies were most abundant, the granules were absent from the leukocytes. Curtis does not speculate as to whether these were altered white corpuscles or a foreign cell entering the blood from the tissues. Hayem[33] concludes that there is no essential change in the leukocytes during starvation, at least in dogs.

Considered numerically as to both total and differential estimations, the following results have been obtained from studies of fasts in man. In both Cetti and Breithaupt[25] a moderate decrease was observed during, with a considerable rise the first few days following fast. Senator[45] concludes that there is a lively new formation of leukocytes on refeeding. Luciani[14] records a marked diminution in the early period of Succi's thirty-day fast, dropping from 14,536, the count on the first day, to 861 on the seventh. The count then rose to 1,550, where it remained with slight fluctuations due to concentration and dilution of blood until the twenty-ninth day. He attributes the marked diminution to the digestive action of trypsin, which evidently enters the blood as such during the cessation of intestinal digestion. He bases this theory on the work of Albertoni, who by intravenous injection of trypsin secured almost complete disappearance of leukocytes. The trypsin apparently has no effect on the erythrocytes. It is quite possible, also, that there may exist in the early days of the hunger period some special destructive condition, evidenced also by the loss of hemoglobin. Two other factors at work, he argues, are, first, the disappearance of the lymphocytes, they no longer being required to alter the assimilated products of digestion in the blood plasma (after the work of Schaeffer, Hofmeister and Zawarykins) ; secondly, the leukocytes may have lost their *Wanderlust*. There would then not only be a failure of "outwandering" from the blood, but of "inwandering" from the tissues as well and the latter factor would exert the greater influence on the number, because there would be less active replacement of the cells lost by death. The white cells practically disappeared from Succi's blood during his fourth fast, until late, when small and ill-formed corpuscles were found. Tauszk[31] noted a decrease in total count during one of Succi's thirty-day fasts. This was due to loss of mononuclear cells, including lymphocytes. The

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45. Senator: Bericht über die Ergebnisse des auf Cetti ausgeführten Hungersversucht. Berl. klin. Wchnschr., 1887, xxiv, 427.

eosinophils and polymorphonuclears were increased. There was little change in the white cell content of Martin's[35] blood during his nine-day fast, except that on the second and ninth days they rose to 10,000. There was a very slight progressive loss in the polymorphonuclears, while the lymphocytes were increased somewhat on the sixth and ninth days. There was no differential count made on the second day to explain the total rise on that day.

The result of the two examinations of Gayer's[24] blood are given in Table 1. The striking points in this case are the very low total count on both occasions, the increase in the small and the drop in the large lymphocytes at the end of the fast. No explanations are offered by Wile.

TABLE 1.—EXAMINATION OF GAYER'S BLOOD

Day	Total Whites No.	Poly. Pct.	L. Lymph. Pct.	S. Lymph. Pct.	Eosin. Pct.	Baso. Pct.
18	2,600	54	12	30.4	1.6	2.0
30	2,800	51	5.4	42	1.0	0.6

In Dr. Benedict's[36] subject, during the first fast of seven days, there was a progressive diminution until the last day, when there was a slight rise. After an interval of nineteen days, there was a second fast of four days. During this period, a gradual rise, amounting to about 2,000, was noted. The polymorphonuclears averaged high during both fasts, but at no time could the number be considered distinctly pathological. The small lymphocytes averaged low. The large lymphocytes were high during the last two days of the first period and throughout the last. The eosinophils were low and the basophils high in both fasts. Charteris[23] in 1907, found a moderate leukocytosis, reaching 14,000 on the sixth day from 5,300, the count before the fast. He noted further a gradual increase of the eosinophils to 7 per cent., a condition that had never been noted before in human blood during inanition. His subject went fourteen days without food, receiving a constant quantity of water, 1 liter a day. Penny's[37] blood showed also a slight leukocytosis with a return to normal at the end. The noteworthy features of the differential counts are the high polymorphonuclear percentage, the very marked falling off of the lymphocytes and the increase in the large mononuclears. Reyne[34] could demonstrate no influence on the leukocytes in his dog that fasted for 25 days. Howe and Hawk[46] studied two men during seven-day fasting periods with uniform water allowance. There was an increase in the polymorphonuclears at the beginning followed by a decrease to below normal by the end of the fast. The small lymphocytes presented the reverse picture, while the large lymphocytes increased during the early days. One subject showed a moderate increase in eosinophils. The blood of both men returned to normal after several days of diet.

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[46] Howe, P. E., and Hawke, P. B.: Fasting Studies; ix. On the Differential Leukocyte Count during Prolonged Fasting, Am. Jour. Physiol., 1912, xxx, 174.

Even in animals where conditions can be comparatively readily controlled, there has been a striking lack of harmony in the findings. Rabbits and dogs have been the animals of most frequent choice and Okintschitz's[47] report of his work on the leukocytes of the former in 1892, is one of the earliest. He was concerned only with the differential variations. In normal rabbits the eosinophils constitute about 50 per cent., lymphocytes 25 per cent., large round cells and polymorphonuclears each 12.5 per cent. The animals were allowed no water. He found diminution of the lymphocytes and polymorphonuclears and an increase in eosinophils and large round cells. The polymorphonuclears show the most marked diminution and on refeeding they are increased, seeming, therefore to be the form most affected by food. The disturbance in blood-picture is still evident even when the animals have almost completely regained their body weight. Hayem,[33] much earlier, in 1882, could see no difference between the variations of the leukocytes in the dog studied by him during its twenty-five-day fast and those which occur under normal .circumstances.

Argaud and Billard[48] found about the same alterations in the blood of the two rabbits they studied as did Okintschitz. They report a marked hypoleukocytosis with an inversion of the formula, there being present three mononuclears to every polymorphonuclear. The recovery in these animals, however, was more rapid, for in a few days the blood-picture had resumed the normal. Källmark[44] also studied rabbits during periods of complete starvation varying from seven to fourteen days. He noted a primary fall in lymphocytes and polyneutrophils, followed by a rise until the seventh day, slow in the latter form, more rapid in the lymphocytes. In the longer fasts this rise was followed by fluctuations, none of which, however, went as high as the normal counts. The basophils showed a marked rise on the third day of the longer fasts. On refeeding, the polymorphonuclears showed a more rapid rise than the lymphocytes, duplicating the experience of Okintschitz. Källmark concludes that the lymphocytes are supplied in greater abundance during inanition, most probably by the thymus, which in so doing atrophies. The primary fall and the postinanition rise in the leukocytes occur, he believes, before compensation for the disturbances of equillibrium has been established or the organism has adapted itself to the altered conditions. When this has been accomplished, the changes in the blood are not so much different from those noted under normal circumstances.

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[47] Okintschitz, E.: Ueber die Zahlenverhältnisse verschiedener Arten weisser Blutkörperchen bei vollständiger Inanition und bei nachträglicher Auffüterung (Versuche an Kaninchen) Arch. f. exper. Path. u. Pharmakol., 1892-1893, xxxi, 383.

[48] Argaud, R., and Billard, G.: Inversion de la formule leucocytaire sous l'influence de l'inanition, Mém. Soc. de biol., 1911, lxx, 746.

Rieder[49] reports finding a marked hypoleukocytosis in the dogs he studied in 1892. Liuboumdrow[19] found that the leukocytes of his fifteen dogs decreased gradually at the beginning of their fasts until a loss of 20 per cent. body weight was reached. A gradual rise was then noticed, except in six of them, frequently reaching normal. The lymphocytes showed a diminution persisting to the end, most marked early, dropping from 15 per cent. to 3 per cent. or less. The monocytes reappeared to a certain extent, after the primary fall. The polymorphonuclears were proportional throughout to the total count. Eosinophils appeared early in those animals that did not show them before fasting, and in most cases there was an increase of seven or eight times, which lasted until a loss of from 10 to 30 per cent. in body weight had occurred, when they began to diminish. By the end of the fasts, they had disappeared altogether.

Poletaëw[30] and Reyne[34] both observed great variations in the number of white cells in the dogs they studied. The former concluding that there was evidently a diminution in all the forms until a loss of from 30 per cent. to 40 per cent. in weight and then an increase toward the end, of the younger elements, including lymphocytes. Uskow[30] interprets these results thus: In the beginning, the entrance of young leukocytes into the blood is retarded, as is also the transition of the young into ripe forms. In the latter period, however, the lymph tissue, probably stimulated into increased activity by the products of degeneration, sends more cells into the blood and further, there is probably a more rapid development of the young forms already present into ripe cells. Keuthe[50] noted a decrease in polymorphonuclears and an increase in lymphocytes during the first days and a reversal of this relation in the latter days of fasting. Paschutin,[12] on the other hand, concludes that the fast has practically no effect on the leukocytes, that they show very little alteration.

Howe and Hawke[46] observed the following changes in four dogs receiving only a constant quantity of water: Three of them, going 117, 15 and 30 days respectively, showed a decrease in polymorphonuclears with an increase in the small lymphocytes. The basophils, eosinophils and transitional forms showed no noteworthy changes. The fourth dog, fasting for forty-eight days, was already anemic. His blood presented the reverse picture, the polymorphonuclears increased, the lymphocytes decreased. An eosinophilia that developed early disappeared. Two of these four animals showed an increase in large lymphocytes while the other two a fairly constant decrease in the same variety of cell. During repeated periods of fifteen and thirty days fasting in these dogs, the results were quite different, all the forms remaining practically constant, save the large lymphocytes.

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[49] Rieder: Beiträge zur Kenntnis der Leukocytose, Leipsic, 1892.

[50] Keuthe, W.: Ueber die funktionelle Bedeutung der Leukocyten im zirkulierenden Blute bei verschiedener Ernährung, Deutsch. med. Wchnschr., 1907, xxxiii, 588.

With regard to the question of hibernating animals throwing some light on the findings during simple starvation, the work of Hansemann[51] is interesting. When the animals were killed during their hibernating state and the various organs examined, no evidence of cell division could be found. He concludes that the physiologic cell division occurs as a result of the mechanical wearing out of the tissue. If this is eliminated, as it is under these circumstances when muscular and digestive activity and the general vital processes are practically in abeyance, there is no stimulus for cell division. The reduced activity, the absence of intestinal digestive processes or products and the presence of perverted products of parenteral digestion during fasting, no doubt would be the important factors in influencing the blood picture. Argaud and Billard[48] examined the blood in hibernating dormice and only a few monocytes were found after careful search, the other forms having apparently entirely disappeared. Valentin, quoted by Paschutin,[12] had the same experience, finding only a few white cells. He explains their absence to the lack of lymph, which, he asserts, introduces the leukocytes into the blood.

Interesting also in view of the findings in some of the cases of inanition quoted above are the changes noted in bone marrow by Roger and Josué.[52] In rabbits that were completely starved for six or seven days, the marrow showed the presence of many giant cells. Neutrophilic granular myelocytes predominated, though there were many polymorphonuclear cells. Eosinophilic cells were rare. The fat was largely replaced by a granular, albuminoid substance, not mucin. On refeeding, the eosinophils were even less in evidence, but there were many very large giant cells and numbers of nucleated red cells, some of them polynuclear. Not before twenty-four days of feeding did the marrow return to its normal state. This picture is not altogether consistent with the blood-findings reported, especially the eosinophilia described by Okintschitz[47] and the scarcity of polymorphonuclears observed by Argand and Billard.[48]

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[51] Hansemann, D.: Ueber den Einfluss des Winterschlafes auf die Zellteilung, Arch. f. Physiol., 1898, v and vi, 262.

[52] Roger, J., and Josué: Des modifications histologiques de la moelle osseuse dans l'inanition, Compt. rend. Soc. biol., 1900, lii, 417.

As already stated, the thymus has been found to atrophy during inanition. Källmark[44] noticed diminution not only in the size of the organ, but in the number of mitotic figures, an evidence of its inactivity. He quotes von Friedleben as having made the same observations as early as 1858, Hammar in 1905 and von Jonson in 1909. These are the only references that could be found to the histological appearances of the hematopoietic organs during starvation. This is a neglected feature of the subject that would seem to offer a profitable field for investigation.

PHYSICOCHEMICAL CHANGES

Specific Gravity

The question of influence of food and drink and the abstinence from them on the density of the blood has been rather frequently the subject of investigation. We find that as early as 1834, Thacrah[53] noted an increase in the specific gravity during hunger periods. J. Davy[54] obtained the same result by depriving his subject only of water and Nasse,[29] starving dogs but allowing water, found a decrease in specific gravity after three or four days, but that by the eleventh day, the blood had returned to or even exceeded its normal density.

Liuboumdrow,[19] using the pyknometer, detected fluctuations in density as marked, comparatively, as those noted in the number of red cells, but a complete agreement between specific gravity and erythrocyte count was not found. Castellino,[55] studying starving rabbits, found an increase in density and at the same time a decrease in the serum content of their bloods. Popel[56] also reports an increase, though a slight one. He studied both rabbits and dogs, using Hammerschlag's method. In the former the increase did not exceed 1.6 per cent. and it was still less in the dogs. After ligation of the ureters, there was the slight fall of 9.11 per cent. from normal in rabbits, while the dogs showed a rise of 0.72 per cent., a rather unexpected result, if taking only the water content of the blood into consideration. London's[11] findings do not agree with those above. He also used Hammerschlag's method, but reports a slight diminution in the rabbits that starved for from five to fourteen days, the average dropping from 1.048 to 1.043. The animals in both the above-mentioned series were deprived of water.

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[53] Thacrah: An Inquiry into the Nature and Properties of the Blood as Existent in Health and Disease, London, 1819-1834.

[54] Davy, J.: Physiological and Anatomical Researches, London, 1839.

[55] Castellino, P.: La suscettibilitá infettiva nella inanizione lenta, Riv. d'ig. e san. Pub. 1893, iv, 461.

[56] Popel, W.: Sur les variations de la densite du sang dans le jeûne absolu, simple, ou compliqué de la ligature des uretères; from the laboratory of general pathology, Prof. S. Lukjanow, Arch. d. sc. biol., 1895-6, iv, 354.

There was evidently considerable fall in Martin's[35] blood, for while no preliminary estimation was made, on the sixth day of his fast the specific gravity was 1.026; on the eighth there was a rise to 1.031 and on the ninth and last day it had dropped to 1.021. One week after breaking fast it was 1.043, still very low if we consider the normal to be 1.059 to 1.060. Lloyd Jones is quoted by Lyonnet[57] as finding, on the tenth day of one of Succi's fasts, a specific gravity of 1.061 that rose to 1.063 the thirty-ninth day.

Lyonnet,[57] speaking of the influence of food and drink on the specific gravity, holds that there is usually though not invariably a diminution after the intake of water, the change being but very temporary. Abstinence from all liquid causes an increase that is not so marked, however, as one would suppose. (In this he is quoting Lichtheim). Food apparently has some effect, in that after meals there is a decrease to be found that lasts for an hour or so.

Coagulability

Very little mention is made in the literature of the influence of inanition on the coagulation time. Arnold[17] and Collard de Martigny,[17] both noticed that the clot was larger than usual in relation to the amount of serum and the latter in 1850 found a decrease in fibrin content. Jones[17] also noted that the water and fibrin decreased more rapidly than the solid constituents. Källmark[44] noticed that in rabbits, after the fifth or sixth day of starvation, the blood coagulated more rapidly, but no estimation of the time is given. Vierordt[58] was among the first to refer to this feature of the subject, having made the observation in 1878 that an acceleration of the process occurred as a result of starving. Tria[59] reports quite recently that he could detect very little variation during short fasts in rabbits and dogs. Valentin[9] noticed a marked retardation of coagulation in hibernating animals.

No report of the specific estimation of coagulation time during inanition in man could be found. Dr. Wile[24] reports that on both examinations of Gayer's blood made on the eighteenth and thirtieth days there was apparent decrease in the platelets, but that coagulation was accelerated. He says of the last examination that "the blood was thick, dark red and did not flow easily." Aside from such general conclusions without data to demonstrate them, the only clue as to what might be expected in man are a few observations that have been made relative to meal times. Coleman[60] found the longest coagulation time one hour after the principal meal and the shortest before breakfast. Cohen,[61] using the method devised by himself determined that the average time before meals was 7 1/6 minutes and after meals 9 minutes, while Mercier, quoted by Cohen in the above-mentioned article, constantly found the coagulation more rapid after meals than before and Addis[62] asserts that food has no influence on the process. Cohen[61] quotes A. E. Wright as crediting fluids with a greater influence on coagulability of the blood than food, but that hunger does retard the process, a view not upheld by the observations of Coleman and Cohen. Increased consumption of liquids lengthens the time and withholding them has the opposite effect.

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[57] 57. Lyonnet, B.: De la densité du sang, sa détermination clinique, ses variations physiologiques et pathologiques. Paris, 1892, p. 73.

[58] 58. Vierordt, H. C.: Arch. d. Heilk., 1878, xiv, 193.

[59] 59. Tria, P.: Propriétés chimico-physiques du sang durant l'inanition. archiv. ital. de biol., 1911, lv. 49.

Immunity

There have been a few studies made of the effect of starvation on immunity in general and the immune body content of the blood specifically, but the data are scarcely sufficient to warrant definite conclusions. In 1890 Canalis and Morpurgo[63] studied the effect on the natural immunity which pigeons exhibit toward anthrax. They were found constantly to lose this resistance if the fast were begun immediately after the injection of the organisms, or a day or so before. They regained it, however, on refeeding if the inanition period had not been too long. The same natural immunity possessed by chickens[64] was not lost unless they were starved for more than 8 days. If starved before inoculation, they proved more susceptible. These workers were unable to make rats susceptible to anthrax by starving. P. Castellino[55] concluded from his studies on rabbits in 1893 that there was a diminution in resistance to infection. A few years later, 1899, Meltzer and Norris[65] could demonstrate no difference in the bactericidal action against the typhoid bacillus of the blood of starved, under or overfed dogs. Roger and Josué,[66] on the other hand, having observed an increase in the resistance to the colon bacillus in fasting rabbits suggest that some possible benefit may be derived from fasts. This is the only bit of experimental evidence speaking for the value or advisability of this procedure.

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[60] Coleman, C. J.: The Coagulation of the Blood and the Effects of Certain Drugs Upon It, Bio-Chem. Jour., 1906-7, ii, 184.

[61] Cohen, M. S.: Coagulation Time of the Blood as Affected by Various Conditions, ARCH. INT. MED., 1911, viii, 684.

[62] Addis, T.: The Coagulation Time of the Blood in Man, Quart. Jour. Exper. Physiol., 1908, i, 305.

[63] Canalis, P., and Morpurgo, B.: Ueber den Einfluss des Hungers auf die Empfänglichkeit für Infectionskrankheiten, Fortschr. d. Med., 1890, viii, 693.

[64] Canalis, P., and Morpurgo, B.: Fortschr. d. Med., 1890, viii, 729.

[65] Meltzer, S. J., and Norris, C.: On the Influence of Fasting upon the Bactericidal Action of the Blood, Jour. Exper. Med., 1899, iv, 131.

Charteris[23] noticed a wide daily variation in the opsonic index of the blood of his human subject during the latter's fourteen-day fast, but as he obtained a similar result with his own blood, he was led to conclude that the changes during fasting were not significant, due, rather, to the use each day of a fresh emulsion of bacteria. Martin's[35] blood, however, showed a gradual lowering of the index, returning to normal four days after the fast was broken. In this case the *Staphylococcus aureus* was used.

Among the studies on other properties of the blood are to be mentioned those of Tria[59] with reference to the viscosity and electroconductivity in rabbits and dogs. He found little alteration, some decrease in both early. He concludes from his entire study that the body is able to compensate pretty well for the disturbances in nutrition, thus permitting long fasts without real serious consequences. A decrease in alkalescence was noticed by Tauszk[31] in Succi's blood and Benedict reports the same change in his subject.[36] A very moderate decrease was also observed by London[11] in his eight rabbits.

THE AUTHOR'S OBSERVATIONS ON LEVANZIN'S BLOOD

The examinations were concerned principally with the red and white cell and hemoglobin content. There was an attempt made to determine the opsonic index, but the subject was so far from an incubator and centrifuge that it was found impossible to obtain anything like accurate results, so this feature was abandoned, though still recognized as one of the most important lessons to be learned from the blood. The coagulation-time and specific gravity were also investigated.

For three days just preceding the fast, Levanzin's blood was examined to determine a normal picture with which to compare the results later obtained. During the fast, with the exception of the first day and three days scattered through the period, daily examinations were made and in addition, on the first and third day of refeeding. The time of the day did not vary more than half an hour throughout, all the specimens being obtained between 10 and 10:30 a. m., so that there was a constant relation to the general routine of the subject's daily activities, that is, immediately after he had finished with the respirometer experiment, been weighed, washed his face and hands and climbed the short flight of stairs to his balcony. It was his habit to take about half a glass of water before submitting to the lancet-prick. It is well to mention here that after the first three days, the quantity of water ingested daily was uniformly 750 c.c.

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[66] Roger, J., and Josué: Influence de l'inanition sur la resistance à l'infection colibacillaire, Compt. rend. Soc. biol., 1900, lii, 696.

The Thoma-Zeiss apparatus was used for counting the red and white cells and smears for the differential counts were stained by the Wright method. For the estimation of the hemoglobin percentage, the Tallqvist[68] and Sahli apparatuses were used.

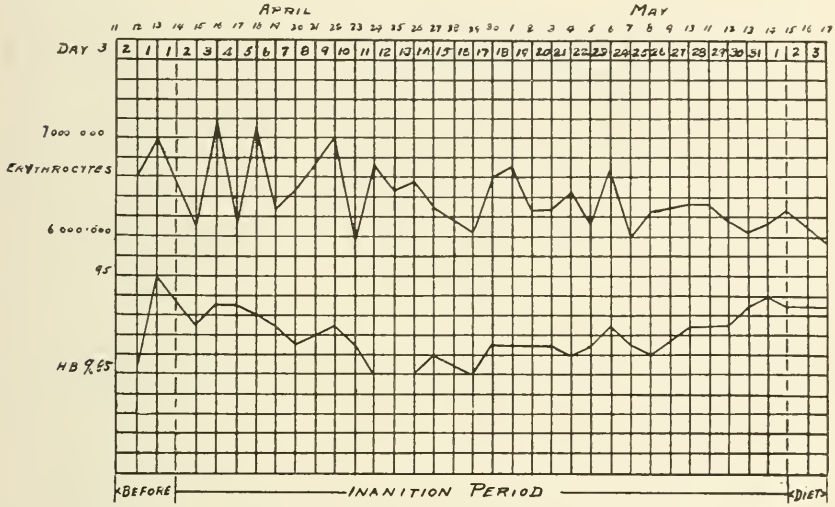

Fig. 1.—Relation of hemoglobin to red cells in fasting subject.

The results of these examinations are contained in Table 2. They are correlated and presented more graphically by Figure 1, to show the relation of hemoglobin to red cells, and by Figure 3, to show the relation of the total white count to the differential. To avoid confusion of curves, the transitionals, eosinophils, basophils are plotted separately in Figure 4 and the scale enlarged. In Figure 2 the composite curve of the polynuclears, that is, neutrophils, basophils and eosinophils and composite curve of the mononuclear cells, large and small lymphocytes, transitionals and monocytes are presented for comparison.

Erythrocyte.—The subject's normal count apparently was high. The three preliminary estimates range well above 6,000,000. It maintained this high figure throughout the test, going below only on two occasions, the tenth day of the fast and the third day following. In

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[68] Tallqvist, T. W.: Ueber die Anwendung des Filtrirpapiers im Dienst der praktischen Haematologie, Berl. klin. Wchnschr., 1904, xli, 926.

TABLE 2.—Daily Protocol of Blood-Count in Fasting Subject

Day	Hb. Pct.	Erythrocyte	Leukocyte	Poly. Neut.	S. Lymph.	L. Lymph.	Monocyte	Trans-itional	Eosin.	Basop.
BEFORE FASTING PERIOD										
3	85	8,984,000	5,900	67.5	23.5	2.5	4.5	1.5	0.5	0.0
2	85	6,632,000	6,400	66.0	22.0	1.5	7.5	2.0	0.5	0.5
1	95	7,000,000	6,000	59.5	32.0	3.0	4.0	0.0	1.5	0.0
FASTING PERIOD										
2	90	6,100,000	8,400	73.0	20.0	3.0	3.0	0.5	0.5	0.0
3	92	7,200,000	12,400	79.0	16.5	0.5	3.0	0.5	0.5	0.0
4	92	6,120,000	8,400	66.0	24.0	4.0	4.0	1.0	0.5	0.0
5	91	7,170,000	9,400	68.0	26.5	2.0	3.0	0.5	0.0	0.0
6	90	6,250,000	8,600	67.5	19.0	4.5	3.0	5.0	0.5	0.5
7	88	6,450,000	7,000	60.0	24.0	4.0	3.5	6.5	2.0	0.0
9	90	7,000,000	8,600	66.0	26.0	3.5	1.0	3.0	0.5	0.0
10	88	5,950,000	7,000	68.5	22.0	3.0	3.0	1.5	1.5	0.5
11	85	6,750,000	8,400	68.0	23.0	2.0	5.0	2.0	0.0	0.0
12	85	6,480,000	7,000	74.0	15.0	2.0	4.5	3.0	1.5	0.0
13	85	6,580,000	6,800	64.0	24.5	7.5	1.0	2.0	0.5	0.5
14	87	6,280,000	7,900	64.0	26.5	4.0	2.5	2.5	0.5	0.0
16	85	6,010,000	9,000	72.5	16.5	3.5	1.5	5.0	0.5	0.5
17	88	6,600,000	6,200	64.5	29.0	3.0	0.5	3.0	0.0	0.0
18	88	6,700,000	6,600	65.5	24.0	6.5	2.0	1.0	1.0	0.0
19	88	6,250,000	6,200	62.5	22.5	5.5	3.5	5.0	0.5	0.5
20	88	6,250,000	6,400	61.0	28.5	3.0	3.5	2.5	1.0	0.5
21	87	6,450,000	6,400	62.5	20.5	5.0	5.5	5.5	1.5	0.0
22	88	6,130,000	6,100	61.0	26.5	3.5	6.5	2.5	0.0	0.0
23	90	6,630,000	6,600	58.5	28.0	4.5	4.5	3.0	0.5	1.0
24	88	6,000,000	6,900	65.0	25.0	2.5	4.5	2.5	0.5	0.0
25	87	6,250,000	6,500	65.5	27.0	1.5	5.0	1.0	0.0	0.0
27	90	6,240,000	5,800	63.0	26.0	3.0	6.5	1.5	0.0	0.0
28	..	6,350,000	7,800	61.5	30.0	1.0	5.5	2.0	0.0	0.0
29	90	6,190,000	6,000	63.5	27.5	1.5	5.0	2.0	0.0	0.5
30	92	6,050,000	6,000	64.5	26.5	2.0	5.0	1.5	0.5	0.0
31	93	6,170,000	8,000	60.0	32.0	2.5	5.0	0.5	0.0	0.0
DIET										
1	92	6,280,000	7,200	70.0	21.5	2.5	4.5	1.5	0.0	0.0
2*										
3	92	5,960,000	6,600	60.5	32.5	3.0	3.0	0.0	0.5	0.0

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* Too ill for examination.

the early part of the fast there was daily variation, ranging under 1,000,000. This became less evident toward the end. The general impression given by the curve is that of a very moderate decrease. there were no alterations in the characteristics of the individual cells as to size and shape and no nucleated reds were found at any time.

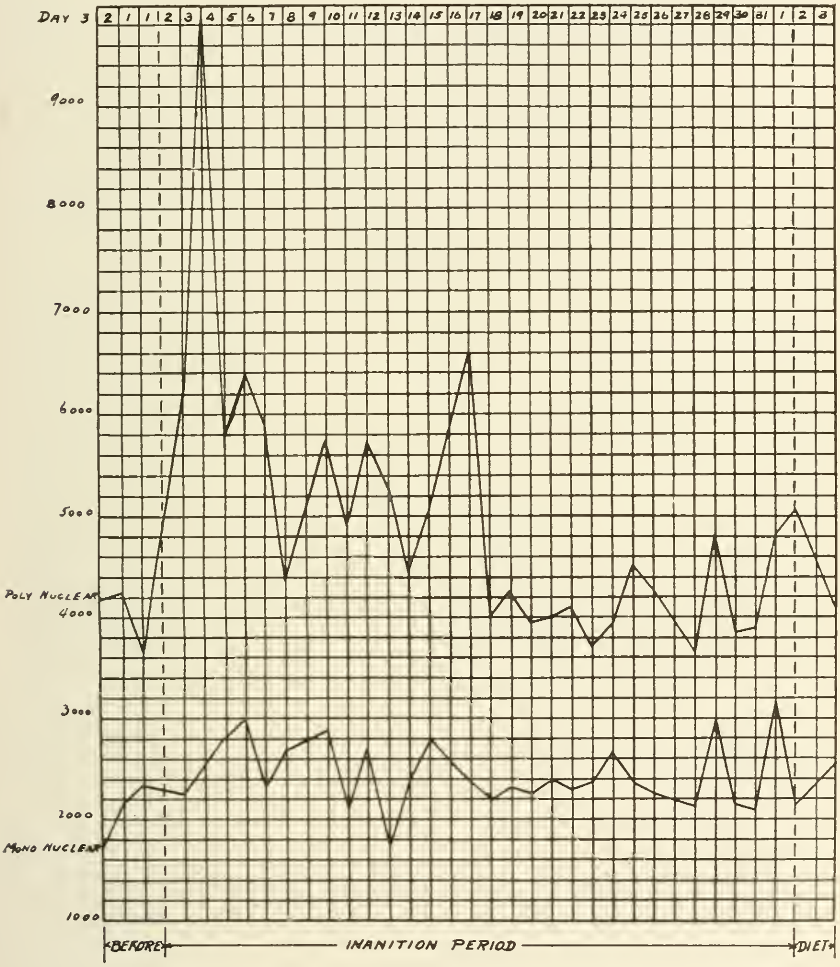

Fig. 2.—Composite curve of the polynuclears (neutrophils, basophils and eosinophils) presented for comparison with a composite curve of the mononuclears, large and small lymphocytes, transitionals and monocytes.

Hemoglobin.—The hemoglobin average ranges rather consistently above 85 per cent., the most marked variations being consistent with those of the erythrocytes. The low period is between the tenth and sixteenth days, from then on showing a very moderate rise.

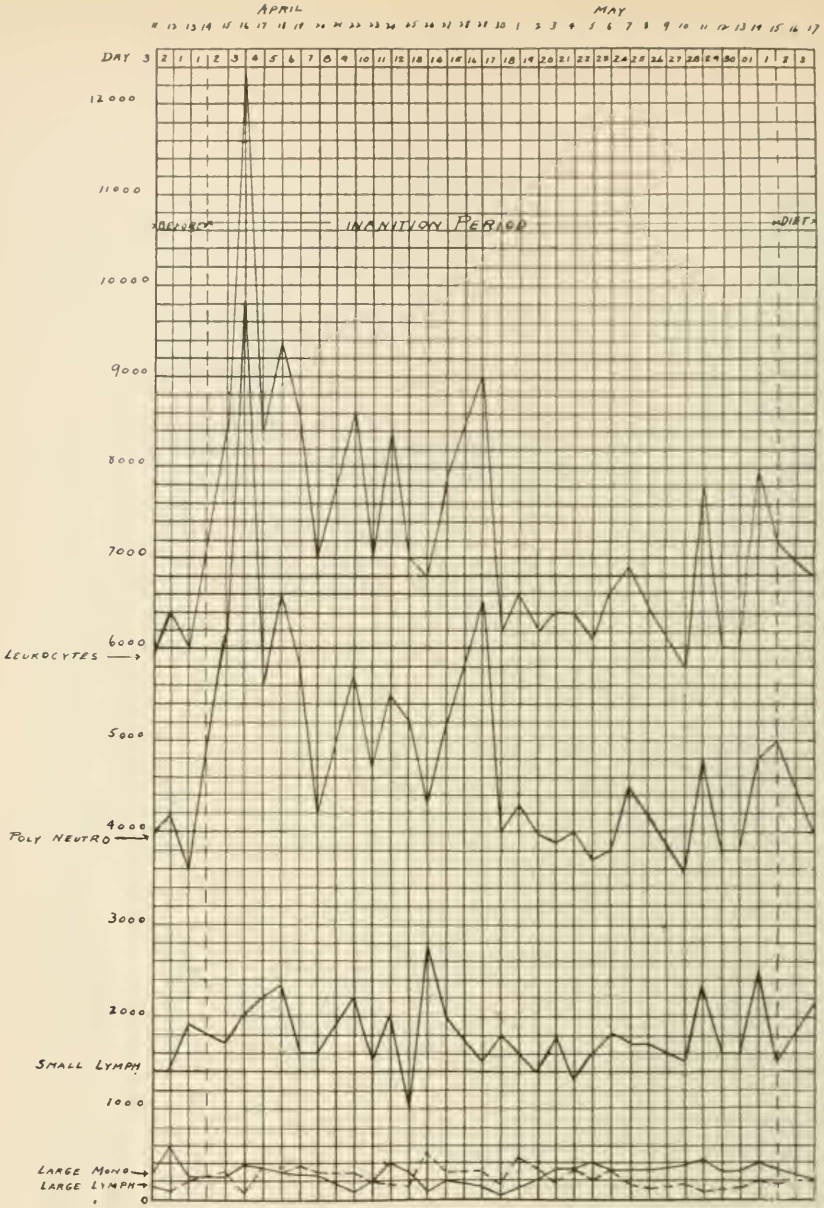

Fig. 3.—Relation of total white count to differential.

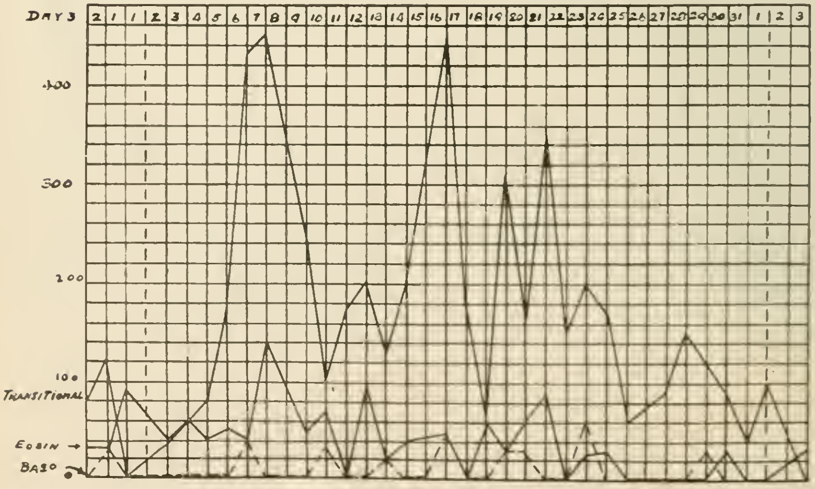

Fig. 4.—Transitionals, eosinophils and basophils.

Leukocytes.—It is in the total leukocyte count that we have the most striking change of the whole series of examinations. There was a rapid rise at the onset of the fast, reaching 12,400 on the third day. On the fourth day, however, it immediately fell to 8,400, after which there was a consistent daily variation of about 1,000 until the sixteenth day, when it reached approximately the preliminary count, after which there will be noticed a more marked daily variation. It was possible to continue the examinations for only three days following the fasting period so that the count had not settled down to normal when the subject went out from under observation.

Polymorphonuclear Neutrophil.—These ran throughout quite consistently with the total count, and the marked variations in this latter were quite apparently due to the change in polynuclear content.

Small Lymphocyte.—There is a number of fluctuations in the count of this cell, except during the period between the fourteenth and twenty-seventh days, when they were quite constant, as were also the total and polymorphonuclear counts. It will be noted that the majority of the rises and falls are the opposite of those seen in total and polymorphonuclear curves. This is particularly the case on the second, fourth, thirteenth, fourteenth, sixteenth, seventeenth, twentieth and twenty-first days of fast and the first day of refeeding. It seems safe to conclude, therefore, that these fluctuations are only relative, being due really to the fluctuations in polymorphonuclears and that their number was practically constant throughout the fast. The other forms present no distinctive features except the transitional, which was subject to several rises, namely, on the seventh, sixteenth, nineteenth and twenty-first days, when they were about 5 per cent. Only an occasional eosinophil was found during the last ten days of the fast and they had not returned to their usual number when examinations were discontinued. By examination of Figure 2 there appears to be a very slight increase in the combined mononuclear cells throughout the fasting period, the average being raised by the fluctuations in the transitional form, for the other types, that is, monocytes, large and small lymphocytes, are practically constant throughout except the variation already noted in the latter.

Coagulation-Time.—Toward the end of the second week of inanition, it was noticed that the blood coagulated more rapidly than it had during the earlier days. It became more noticeable each day so that if the mixing pipets were not filled very rapidly, the drop would coagulate, or the blood would clot in the tubes. It is certain that this was not due to any physical alteration of the patient's environment. The temperature of the balcony where the subject stayed and where the estimations were made was practically constant. This is a very important factor, for most experimental evidence goes to show that variations in temperature have decided influences on the coagulation time.[69, 70]. On the seventeenth day the estimations of the time were begun. Until the twenty-fifth day the McGowan method was used. Capillary tubes of uniform caliber were filled with blood escaping after the specimens for the other examinations had been obtained, practically always the same relative drop, the third. Small sections were broken off at intervals of from ten to thirty seconds, and when a fine filament of fibrin was observed between the carefully separated ends of the tube and fragment, the time elapsed since the appearance of the drop used was taken as the reading. For the remainder of the examinations, the Boggs[71] apparatus was used.

TABLE 3.—Protocol of Coagulation Time of Blood

No. Day	Coagulation Time		Remarks
	Levanzin	Control	
17	1′ 5″	3″	McGowan method.
18	1′20″	2′50″	McGowan method.
19	1′20″	2′	McGowan method
20	1′ 5″	.....	McGowan method.
21	55″	1′55″	McGowan method.
22	1′20″	.....	McGowan method.
23	1′ 5″	1′35″	McGowan method.
24	1′ 5″	4′20″	Boggs used on control.
25	.....	4′50″	From this day on Boggs used on both.
27	3′20″	4′+	
28	2′50″	.....	
29	2′20″	.....	
30	1′50″	3′30″	
31	2′30″	.....	
First diet	2′45″	6′20″	

The figures as obtained by these two methods, together with those found in a normal individual examined on the same days, with the same methods and under approximately the same conditions, are collected in Table 3. The last record on the control was made in a temperature at most 2 degrees C. cooler than that of the subject's environment, but a deduction of two minutes as correction for this still leaves the time distinctly longer than that obtained on the same day in Levanzin's blood. A comparison of the two series of results

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69. Hartman, J.: Zur Frage der Blutgerinnenzeit, München. med. Wchnschr., 1909, lvi, 796.

70. Rudolf, R. D.: Tr. Assn. Am. Phys., Philadelphia, 1910, xxv, 504.

71. Boggs, T. R.: Johns Hopkins Hosp. Bull., 1904, xv, 174.

will demonstrate a distinct increase in the coagulability of the starving man's blood, more noticeable toward the end of his fast.

Specific Gravity.—The specific gravity was determined on only two occasions, the first while the subject was eating his first meal after fasting and the second on the third day of refeeding. On the former occasion it was 1.0612 and on the latter, 1.0618. The estimations were made by the Hammerschlag[72] method, the specific gravity of the mixture of chloroform and benzol being determined by the pyknometer. As no figures were obtained either before or during the fast, these two examinations are of little value except that from them it may be assumed that there is a very slight increase in density, taking 1.059 to 1.060 as the average normal specific gravity.

DISCUSSION AND CONCLUSIONS

The results of the preceding studies are conspicuous rather from the absence than from the presence of striking alterations in the blood-picture. Really the only prominent features are the early rise in polymorphonuclear neutrophils and the decrease in coagulation time. The leukocytes, that is, the neutrophils at least, are the most sensitive of the blood-cells to changes in body conditions and we know that apparently slight disturbances—a cold bath, for example—will call forth a recognizable increase in these cells. They seem always to be on the alert, ready at the least evidence of disturbance to rush forth in defense of the organism. It is scarcely to be wondered at, therefore, that in response to such an unusual condition as starvation there should be an outpouring of the reserve supply, at least for a day or so, or until the organism has had an opportunity to adapt itself to the altered conditions. The variations in water content of the blood cannot be considered a factor in this rise, involving as this does only the one form of cell. The only explanation that suggests itself therefore, and frankly not a particularly scientific one, is this alertness of the polymorphonuclear and its constant readiness to be on the defense for the organism. The products of the somewhat perverted metabolism may excite them into this early activity and later fail to do so, but there is no evidence to prove this supposition. It is not easy to understand why they should respond to toxic products in the early days and not the later as well, unless we assume they acquire a tolerance for them, which seems improbable when we compare the reaction to infections, in which their fight is evident throughout the disease if the organism is to conquer.

As to the effect of the starving on the total quantity of blood, it does seem evident that there are fluctuations at least in the first two weeks. It will be noted by comparing the curves of the white and red cell-counts the variations are synchronous, that on the third, fifth, ninth and eleventh days particularly, the noticeable increase in the one are accompanied by equally frank rises in the other. It would seem that this could be due only to variation in water content. The specific gravity would have gone far to prove this point, but unfortunately this was not determined during this period. Taking these fluctuations as indication of variation in water content, it appears that during the middle period of the fast, at least, the equilibrium of intake (including that drawn from tissues) and output was pretty well established.

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[72] Hammerschlag: Wien. klin. Wchnschr., 1890, iii. 1018.

The hemoglobin appears to be particularly resistant, the percentage on the last day being within 2 per cent. of the highest estimation, found on the day before fast began, though there was a moderate decrease during the second ten days.

It is difficult to account for the only other marked change in the blood, that of increase in coagulability. Loss of water could be responsible, but there is no evidence that this occurred. The very slight increase in density at the last, determined by the specific gravity, certainly does not demonstrate a sufficient concentration. There were no estimations of the platelet content made, but it is quite possible that the explanation lies with these. An increase in them could be responsible.

The final conclusions as to the effects of starvation on the blood to be drawn from the results of examinations on Levanzin are:

1. There is a slight actual loss in hemoglobin, more marked during second ten days.

2. There are moderate fluctuations in water content, particularly during first half of period.

3. There is a decided rise in polymorphoneutrophils in the early days.

4. There is an increase in coagulability, especially after the first two weeks.

5. The blood as a whole is distinctly resistant to the effects of uncomplicated inanition, permitting of entire abstinence from food for long periods of time (at least thirty-one days) without deleterious consequences, as far as the blood is concerned.

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THE PATHOLOGIC PHYSIOLOGY OF THE CIRCULATION DURING HEMORRHAGE *

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A number of authors, Marey,[1] Fredericq,[2] Lazarus,[3] Crile[4] and Henderson[5] among them, have presented either a review or their conceptions of the changes produced in the circulation during hemorrhage. Each of these writers has reflected the knowledge available at the time and much that has already been said can merely be reechoed. For a number of years, however, I have been collecting experimental evidence which, in some instances, extends or interrelates the observations already established and, in others, appears to necessitate a revision of current views. Hence, although many problems still await investigation, the attempt is made to present such a picture of the pathological changes in the circulation during hemorrhage as appears justifiable in the light of present-day knowledge. In doing this the hope is entertained that such an exposition may prove of value in interpreting the signs or symptoms that present themselves at the bed-side and in a certain measure also govern the treatment.

I. THE SEQUENCE OF EVENTS DURING HEMORRHAGE

When an artery of considerable size ruptures, the total resistance against which blood is discharged is reduced. Consequently, a fall in diastolic pressure results. The systolic pressure also decreases though not to the same extent as the diastolic. Hence, the pulse-pressure increases (Fig. 1). There are several reasons for this; in the first place, the "effective pressure" in the left auricle,[6] which

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* Submitted for publication, March 23, 1914.

[1] Marey: Circulation du Sang, 1882, p. 192.

[2] Fredericq: Travaux du Laboratoire de Liège, 1885-86.

[3] Lazarus: Diseases of Blood, Nothnagel's Practice, Am. Ed., Philadelphia, 1909, p. 154.

[4] Crile: Hemorrhage and Transfusion, 1909, New York, pp. 63 and 96.

[5] Henderson: Am. Jour. Physiol., 1910, xxvii, 155.

[6] The term "effective pressure" has been applied by Henderson and Barringer (Am. Jour. Physiol., 1913, xxxi, p. 288) to the difference between intra-auricular and intrathoracic pressures and it is this pressure and not the actual auricular pressure which determines the filling of the ventricle.

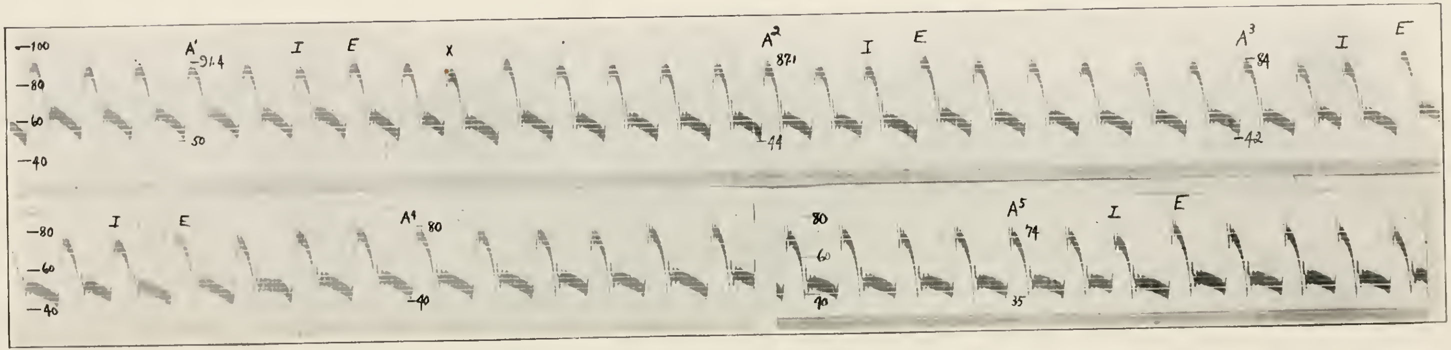

Fig. 1.—Effect of hemorrhage on pulse-pressure taken from carotid by author's optically registering manometer (—— actual size). *I*. Records showing effect of moderate hemorrhage begun at arrow (upper record). Observe that systolic and diastolic pressures vary with inspiration *I* and expiration *E* as compared with the period of intervening quiet. Comparing corresponding waves (A^1, A^2, A^3) in upper curve it is found that systolic and diastolic pressures fall, but pulse-pressure is increased. Later in hemorrhage (lower curves) when both systolic and diastolic pressures have fallen more it is found that the pulse-pressure is decreased.

largely determines the filling of the left ventricle, is not immediately reduced by a diminished supply of blood, hence, the ventricular output is not diminished. In fact, the observations of Johanssen and Tigerstedt[7] indicate that the systolic discharge increases. In the second place, the volume of elasticity coefficient of the arteries becomes greater at lower pressures, hence, the same systolic discharge will give a greater pressure variation. The amplitude of the pulse, which is determined by the pulse-pressure, accordingly becomes large and the shape of the peripheral pulse, which is determined by the low resistance, assumes the collapsing form.

While the left heart is thus maintaining fully a normal discharge and the pulse-pressure is greater, the peripheral flow of blood into the veins is almost immediately reduced. At first, the right auricular pressure is not affected, probably because the reduced volume of venous blood from one region is compensated by an increased flow from another in much the same manner as in clamping the inferior vena cava more blood flows through the external jugular. When the luxus blood-content of the veins has been somewhat reduced, however, this balance of venous flow is lost and the "effective right auricular pressure" is decreased (Fig. 2). Almost simultaneously the volume curve of the entire heart diminishes in amplitude,[8] pulmonary arterial and left auricular pressures fall and the systolic pressure becomes rapidly smaller until the pulse-pressure is less than normal.

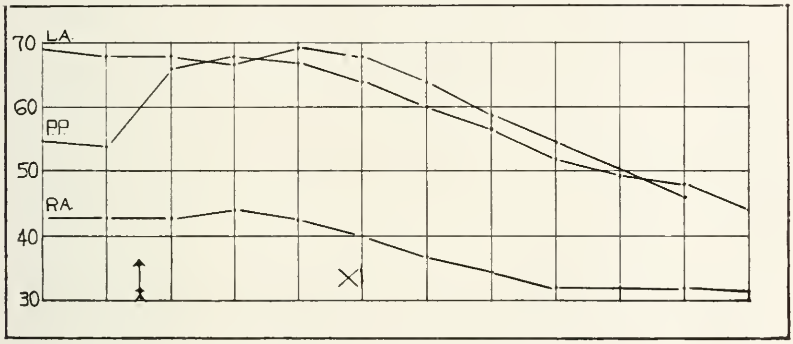

Fig. 2.—Plot from Experiment 377, showing immediate effect of a small hemorrhage at arrow on pulse-pressure (*P.P.*), on effective left auricular pressure (*L.A.*), and effective right auricular presure (*R.A.*) Figures indicate millimeters of mercury when applied to curve of pulse-pressure and millimeters of saline when applied to auricular pressures. Abscissae equal ten second intervals.

The flow through the organs of the body is slowed and the centers in the medulla are stimulated by the reduced blood-flow. Investi-

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[7] Johannsen and Tigerstedt: Skandin. Arch. f. Physiol., 1889, i, 394.

[8] Wiggers, C. J.: The Prognostic Significance of Pulse-Presure Changes During Hemorrhage, Arch. Int. Med., 1910, vi, 281. (See Fig. 1.)

TABLE 1.—Experiment C 18*

Type of Breathing	Auricular Pressure				Intrathoracic Pressure		Effective Pressure (Auricular)			
	Right		Left				Right		Left	
	Insp.	Exp.	Insp.	Exp.	Insp.	Exp.	Insp.	Exp.	Insp.	Exp.
Natural, quiet	55	62	—20	0	— 62	—30	117	92	42	30
Tracheal stimulation (deeper breathing)	50	65	—46	0	— 94	—28	144	93	48	28
Natural breathing (12 min. after)....	60	66	—16	+ 8	— 48	—24	108	90	32	32
Sciatic stimulation (85 seconds)......	66	80	—32	+18	—130	+ 2	196	78	98	16
Apnea (1 min. duration)	66	..	0	....	— 33	....	99	..	33	

* Experiments arranged according to average loss per minute.

gators[9] are not entirely agreed whether an altered blood-flow may affect the bulbar centers without a change in the general gaseous content of the blood, but the increased respiratory movements following the use of pressure-lowering agents has convinced me that such a direct effect exists.

While these dynamic changes are taking place, a series of important chemical alterations occur in the blood. The experiments of Mathieu and Urbain,[10] Bert,[10] Vinay[10] and Finkler[11] all indicate that the loss of red cells from the circulation and the consequent reduction of the oxygen-carrying power, aided by the slowed circulation in the peripheral capillaries lead, first, to a decrease in the oxygen content of the venous and later of the arterial blood, while in neither is the percentage of carbon dioxid materially altered. Some recent gas analyses, performed in this laboratory by Murlin and reported in part by Murlin and Greer,[12] confirm these observations with more recent methods of analysis.

TABLE 2.—Relation of Cardiac Rate to Rate of Loss of Blood

Exp.* No.	Weight kg.	Duration min.	Total Loss c.c.	Percentage Loss	Avge. Loss per min. c.c.	Heart Rate		Pct. Gain
						Before	After	
153	10.0	35	150	1.5	4.3	135	105	
151	10.4	36	208	2.0	5.7	148	112	
176	8.5	37	212	2.5	5.7	99	99	
160	10.1	33	148	1.5	6.4	105	102	
163	6.1	6	60	1.0	10.0	111	135	21.8
178	10.5	35	352	3.5	10.0	108	135	25.0
365	17.0	30	570	3.0	19.0	150	220	46.6
350	9.0	3	90	1.0	30.0	78	102	30.8
359	10.8	5	270	2.5	54.0	105	156	48.6
341	10.0	4	350	3.5	87.0	150	182	21.3
344	17.0	1	170	1.0	170.0	138	156	13.0
364	15.0	1.5	300	2.0	200.0	96	162	75.0

* All figures in millimeters of water.

This reduction in the oxygen percentage of arterial blood operates with the reduced flow to increase the activity of the respiratory center. At first the respirations increase in rate and depth, giving rise for a considerable interval to hyperpnea which is probably attributable to the decreased flow, with perhaps a local concentration of the carbon dioxid in the medulla.[13] The greater depth and rate of respirations thus instituted cause, to judge from the variations in pulse-pressure, a greater average discharge of the left ventricle.[14] No explanation of the mechanism involved is as yet entirely satisfactory. I[14] have previously published records[15] which indicated that an increase in rate and depth of respiration caused no alteration in the right auricular pressure during expiration, but that the more negative intrathoracic pressure during inspiration caused the "effective pressure" to increase. This has been shown by recent experiments to be true of both right and left auricular pressures. Probably no appreciable increase in the blood-supply of the two auricles takes place during expiration until forced abdominal movements occur; but the more frequent occurrence of the more negative inspiratory phase with its greater "effective pressures" favors the *average venous filling* pressure and increases the *average ventricular ejection.*

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[9] Guthrie and Pike: Am. Jour. of Physiol., 1906, xvi, 480; 1908, xx, 451; Eyster, Austrian and Kingsley: Am. Jour. Physiol., 1907, xviii, 413.

[10] Quoted by Fredericq: Travaux du Laboratoire de Liège, 1885-86.

[11] Finkler: Arch. f. d. ges. Physiol., 1875, x, 369.

[12] Murlin and Greer: Am. Jour. Physiol., 1914, xxxiii, 253.

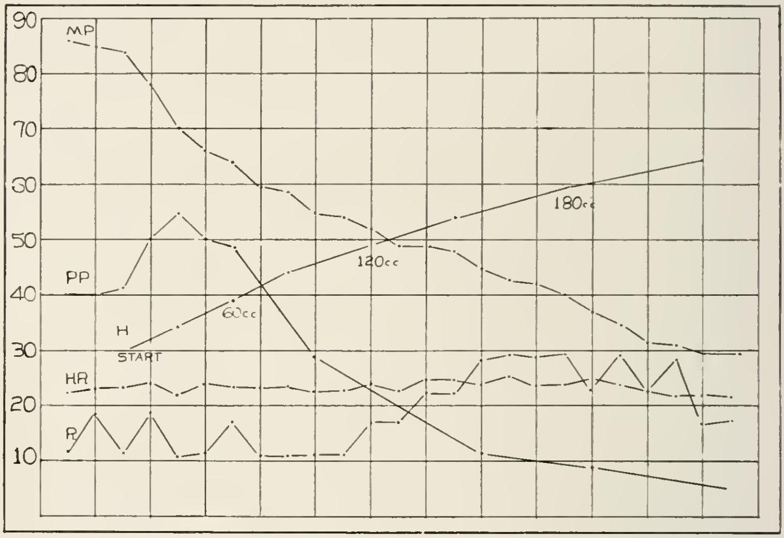

Fig. 3.—Plot from experiment 359, showing effect of a hemorrhage (indicated by line *H*) on mean arterial presure (*M.P.*), pulse-pressure (*P.P.*), heart-rate (*H.R.*) and respiration-frequency (*R*), the latter two estimated for ten second intervals. Abscissae equal ten second intervals.

This tendency of increased respiration to increase the "effective venous" pressure was at first thought to account for the influence of augmented breathing on arterial pressure. That some other mechanism is further concerned, however, is to be assumed, since the arterial pulse-pressure is smallest in inspiration, that is, when the effective pressures in both auricles are highest.

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[13] Paul Bert: La pression barométrique, Paris, 1878, p. 1061.

[14] Wiggers, Eberley and Wenner: Jour. Exper. Med., 1912, xv, 67.

[15] For example, Plate 27, Figure 3, Jour. Exper. Med., 1912, xv, 67.

The altered circulation through the medulla affects also the cardio-inhibitory and vasomotor centers (Plot, Fig. 3). The acceleration of the heart during hemorrhage has been repeatedly observed since Hales,[16] Pawlow, von Leser and others directed attention to this phenomenon and its importance in the maintenance of blood-pressure is generally recognized. The additional work of Fredericq,[17] Hayem[18] and others, has shown that the acceleration results from a depression of the cardio-inhibitory center. Dawson[19] has pointed out, however, that the acceleration is not equally pronounced in all cases, depending on the initial tonus of the cardio-inhibitory center. I have been able to confirm this and, in addition, it appears from the tabulated results that, in a certain measure, the cardiac acceleration is related to the rate at which blood is lost. The data contained in Table 2 indicate that, while the percentage increase in cardiac rate is not related to the actual quantity of blood lost, or to the amount lost in relation to body weight, it is related to the average rate of loss.

Stimulation of the vasomotor center causes an increased contraction of the peripheral blood-vessels during hemorrhage (Sollmann and Pilcher[20]). Such a tendency to increase the total resistance by constriction is antagonized by the reduction in the viscosity of the blood. Cope[21] has shown that, in spite of this, the total resistance remains much increased. Little more than inference seems to support the commonly held idea that the arterial walls contract through vasomotor influence and thus adapt their caliber to the reduced volume of blood.[22] The demonstration of such an arteriomotor reaction by faultless methods would be of great importance. The accommodative diminution in the larger arteries is more probably due to an elastic recoil of the vessels, which is even more favorable, for while the vessels draw together when the pressure lowers, no increased resistance to the flow results, such as would be induced by a general vasoconstriction.

To sum up, the fall of arterial pressures is counteracted by the augmented breathing, an accelerated heart, an accommodative contraction of the larger vessels and by a peripheral constriction leading to an increased resistance in spite of the reduction in viscosity. All the factors enumerated tend not only to keep up the arterial pressure but also to increase the flow through the tissues with the exception of the increased resistance, which tends to diminish the peripheral flow. So great, however, is this single factor that the volume of organs and the venous outflow are always greatly reduced.

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[16] Hale: Hemostatiks, 1733, ii, 2.

[17] Fredericq: Travaux du Laboratoire de Liége, 1886; Arch. du. Biol., 1882, iii, 55.

[18] Hayem: Arch. de physiol norm. et path., 1886, i, 106.

[19] Dawson: Jour. Exper. Med., 1905, vii, 6.

[20] Sollmann, T., and Pilcher, J.: Am. Jour. Physiol., 1910, xxvi, 233.

[21] Cope: Am. Jour. Physiol., 1911, xxix, 137.

[22] See for example, Starling: The Principles of Human Physiology, 1912, p. 1131. Phil. and New York.

If this is so, it may be asked, to what purpose does such a compensatory mechanism exist? Evidently all the pressure-maintaining mechanisms are brought into play so that the brain, with its more feebly innervated vessels, (Wiggers[23]) shall remain perfused by a supply as nearly normal as possible. The experiments of Gaertner and Wagner,[24] Roy and Sherington,[25] Bayliss and Hill[26] and others have shown adequately the direct dependence of the cerebral blood-flow on the general arterial and venous pressures. As arterial pressure is reduced and the cerebral flow decreases, the compensatory reactions not only elevate the general arterial pressure but also divert the blood from the less sensitive tissues to the brain with its vital centers.

It is of further interest to note that the increased rate of cardiac contraction and perhaps the decreased intraventricular tension in themselves supply mechanisms for insuring an adequate blood-flow through the coronaries so that a deficient nourishment and impaired reserve power such as Crile[27] describes does not exist until late in hemorrhage. Many times by the method utilized to establish the innervation of the coronary vessels in the intact circulation, I have observed[28] that the coronary flow after hemorrhage equaled or even exceeded the normal flow, even though the arterial pressure was less than normal (Fig. 4). In this way, it may be explained why the heart is able to maintain a normal contractile power as shown in cardiographic tracings after considerable loss of blood has taken place.

II. THE MECHANISM OF HEMORRHAGE CESSATION

Every hemorrhage, except one coming from a very large vessel, has a natural tendency to be checked long before the loss of blood becomes dangerous. The duration of bleeding depends not so much on the size of the vessels wounded as on the facility with which an efficient clot forms over the opening. When blood is shed, it begins to coagulate on the tissues at a distance from the opening. Having formed a point of adhesion to the tissues, it is added to by accretion until it reaches and covers the wound. The factors which determine the distance of the first clot formation and its development are, therefore directly concerned with the duration of hemorrhage.

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[23] Wiggers: Am. Jour. Physiol., 1908, xxi, 454.

[24] Gaertner and Wagner: Wien. med. Wchnschr., 1887, pp. 602, 639.

[25] Roy and Sherington: Jour. Physiol., 1890, xi, 85.

[26] Bayliss and Hill: Jour. Physiol., 1895, xviii, 356.

[27] Crile: Hemorrhage and Transfusion, New York, 1909, p. 96.

[28] Wiggers: Am. Jour. Physiol., 1909, xxiv, 400.

Other factors being equal, the pressure at the arterial opening determines the distance that blood will be ejected before the force is sufficiently spent to permit the coagulum to form. A very pretty experiment may be performed by allowing a tiny stream of arterial blood to play on a glass slide and impinge against a small elevation cemented to it. The process of clot development may then be watched under a low-power objective. The struggle between the adhesion of agglutinated red cells to the formed clot and the force of the pressure of the stream which attempts to detach them comes clearly before one's eyes. The development of intertwining fibrin threads, making these adhesive masses finally secure and a part of the clot, is of extreme interest. If the pressure of the spurting stream is suddenly increased, unfastened masses may be torn loose and new cells adhere much more slowly. After this fashion the blood-pressure influences the cessation of hemorrhage in the body. Attention may be directed in this connection to the fact not generally emphasized that a clot once formed is called on to resist not the mean but the maximal pressure that exists in the artery. Now, since in conjunction with Eberly and Wenner[29, 30] I have found that the maximal pressure in the systemic as well as in the pulmonary circuit is often entirely determined

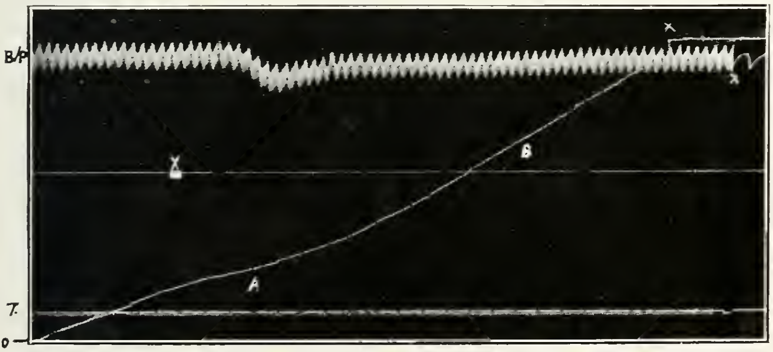

Fig. 4.—Curves from experiment 254, showing effect of a sudden femoral hemorrhage at *X*. The flow through the coronaries indicated by the slope of the curve *V.O.* towards the abscissae at first decreases at *A* but later increases at *B*, in spite of the fact that the arterial pressure (*B.P.*) is less than before bleeding.

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[29] Wiggers, Eberly and Wenner: Jour. Exper. Med., 1912, xv, 174.
[30] Wiggers: Am. Jour. Physiol., 1912, xxx, 233.

by the depth of respiration, it is quite possible that a drug which, in a normally breathing animal, increases the cardiac output and causes a rise in the average mean pressure may, nevertheless, by reducing the dyspnea during hemorrhage actually lessen the maximal pressure in the artery.

The surface on which blood is shed determines the extent of its spread. Thus, the smooth surface on which the intestinal and uterine vessels rupture compares unfavorably with the reticular lung parenchyma and subcutaneous connective tissue. So, also, the spread may be limited by an ulceration or excavation.

The coagulation time directly influences the course of clot-formation. Our conception of the chemical processes has undergone radical modification since the recent work of Howell[31] on this subject. According to this investigator we find, in the circulating fluid, fibrinogen, prothrombin, calcium salts and antithrombin. The last-mentioned substance holds the prothrombin in combination and this prevents its conversion to thrombin. When the blood is shed the disintegrated corpuscles, blood-platelets and the tissues with which blood comes into contact, furnish thromboplastin (chemically a phosphatid resembling kephalin in its properties) which, by combining with antithrombin, liberates prothrombin. This can now be activated by calcium and acts on fibrinogen. Any excess formation of antithrombin (Whipple[32]) or lack of prothrombin such as occurs in hemophilia (Howell[33]) may delay clot formation and interfere with the cessation of hemorrhage.

The formation of clots may be retarded or prevented by muscular contractions of organs. The regular cardiac contraction, as a rule, absolutely prevents any clot formation over wounds of the heart and forms the only serious element in such wounds. The rhythmic peristalsis in the stomach and intestines are very unfavorable for clot formation and the induction of these movements in the intact abdomen by injections of epinephrin may possibly be sufficient to counterbalance any such favorable vascular influence as were indicated by my previous laboratory experiments.[34] On the other hand, the only hemostatic value of atropin resides in its power to inhibit muscular movements and its use *in conjunction with vascular agents* such as epinephrin in hemorrhage from muscular organs is physiologically indicated, especially, as the increasing percentage of carbon dioxid in the blood itself tends to increase the rhythmic contraction of organs.

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[31] Howell: Am. Jour. Physiol., 1908, xxi, 51; 1912, xxxi, 1.

[32] Whipple, G. H.: Hemorrhagic Disease: Antithrombin and Prothrombin Factors, Arch. Int. Med., 1913, xii, 637.

[33] Howell, W. H.: The Conditions of the Blood in Hemophilia, Thrombosis and Purpura, Arch. Int. Med., 1914, xiii, 76.

[34] Wiggers, C. J.: Adrenalin in Intestinal Hemorrhages, Arch. Int. Med., 1909, iii, 177.

III. EVENTS FOLLOWING CESSATION OF HEMORRHAGE

When hemorrhage terminates favorably by coagulation, the volume of blood returned to the right auricle increases with surprising rapidity. This is shown by the ventricular volume curves[35] as well as by the increase in venous pressure quickly following the cessation of hemorrhage (Fig. 5). The immediate cause of this increase is probably found in the prompt rise of arterial pressure following clot formation or application of a hemostat (R^1, Fig. 6) together with the rapid reabsorption of lymph to replete the blood-volume. Incidentally it may be pointed out that this dilution of the blood decreases the number of red cells and diminishes the percentage of hemoglobin (Dawson), thus explaining the posthemorrhagic decrease in both for several days. Practically nothing is yet proved as to the existence of an adaptive contraction of the larger veins in favoring the return of blood, yet the existence of such a posthemorrhagic contraction can be conceived of in view of Mall's[36] interesting contributions to the innervation of large veins.

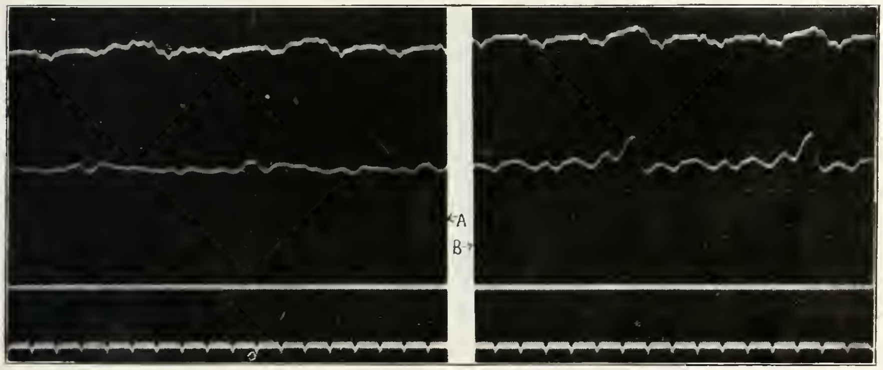

Fig. 5.—Curve showing right (lower) and left, auricular pressures (upper) during hemorrhage (*A*) and 48 seconds after at *B*. One mm. record = 4.3 mm. saline.

In consequence of the better venous supply and greater output of both ventricles, the pulse-pressure increases, for there is little alteration in the peripheral resistance and the increase in the volume elasticity coefficient remains an almost negligible factor. Both systolic and diastolic pressures thus gradually rise, owing to the augmented cardiac output—a change that is mirrored as the secondary rise of mean pressure at R^2 (Fig. 6). The assumption is generally made that this posthemorrhagic increase in mean pressure is further assisted by an additional vasoconstriction; indeed, it is often quoted as the chief or only cause for the pressure recovery. All experimental observations made by the method described by Cope[21] have indicated to me *that vasoconstriction is a gradual process, starting while hemorrhage is still in progress and continuing after its cessation.* Neither experiments nor *a priori* reasoning, however, warrant the assumption that a greater vasomotor reaction after hemorrhage is at all likely to occur, nor is such an assumption necessary to explain this secondary rise.

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[35] Wiggers, C. J.: The Prognostic Significance of Pulse-Pressure Changes During Hemorrhage, Arch. Int. Med., 1910, vi, 281.

[36] Mall: Arch. f. Physiol., 1892, p. 409.

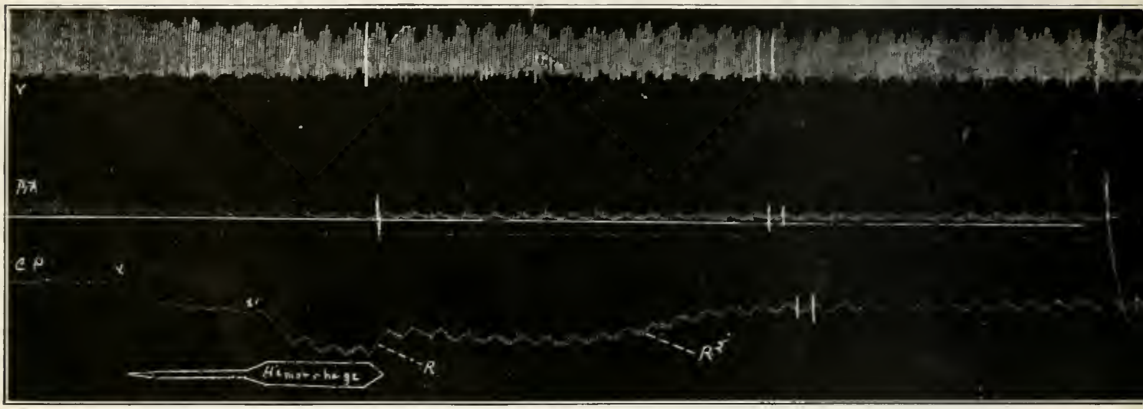

Fig. 6.—Curve from experiment 334, showing effect of femoral hemorrhages on carotid pressure (*P.A.*) pulmonary arterial pressure (*P.A.*) and on cardiometer tracing *V*. *X-X'* small hemorrhage. *X'* to *R'* large hemorrhage. Clamp applied to artery at *R'*. Posthemorrhagic rise at $R.^2$

The rise of arterial pressure, together with the continued dilution of the blood, tends to increase the flow both in the peripheral tissues and in the medulla. The overstimulation of the respiratory center gradually ceases and the respirations become normal again (Fig. 6). The heart continues rapid, however, and the peripheral vessels remain contracted until sufficient regeneration of the red blood-cells has taken place to reestablish normal conditions.

IV. EVENTS IN THE TERMINAL STAGES

If coagulation fails to occur, the time soon comes when the reduction in blood-volume exceeds the possibilities of lymph absorption, to such an extent that the mechanical function of the circulation cannot be efficiently carried out. The amplitude of cardiac contraction then decreases, largely because fluid is lacking to feed the pump. As has been recently pointed out elsewhere,[37] the reduced "initial tension" which this occasions is in itself sufficient to decrease the rate and vigor of the systolic contraction. The reasons for saline infusion or blood transfusion is obvious. Epinephrin also favors the rapidity of cardiac contraction in such cases and so improves the arterial pressure.[37]

The lack of oxygen in the blood soon changes the previous stimulation of the respiratory center to a depression (Fig. 7). Slow and shallow breathing, occasionally marked by a deeper gasp, leads to complete cessation. The "pressor influence" of augmented breathing is removed and the blood-pressure falls rapidly. Asphyxial changes in the circulation terminate the case. The heart-beats are slow but of great amplitude and vigor, thus making a last attempt to elevate the pressure, which is partly successful but soon fails for want of oxygen.

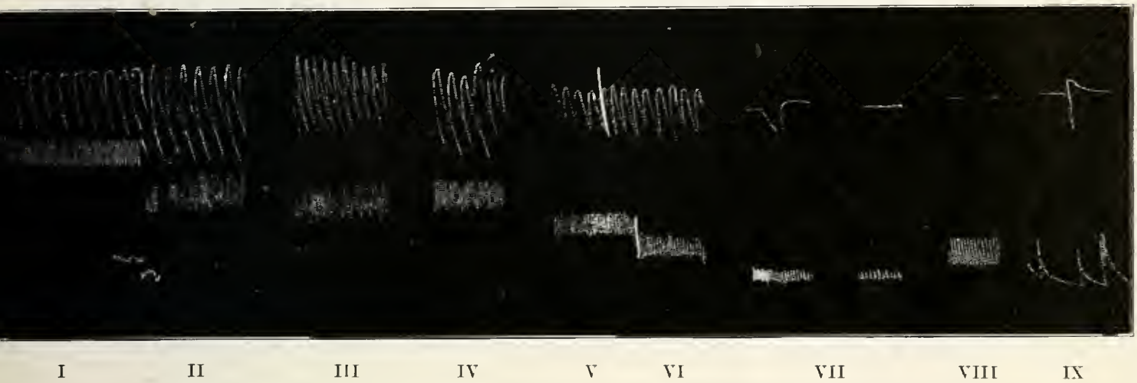

Fig. 7.—Nine segments showing effects on respiration (upper curve) and blood-pressure (lower curve) of a progressive hemorrhage. I. Normal breathing and blood-pressure. II. Deeper breathing with greater respiratory variation in blood-pressure. III. Accelerated deeper breathing. IV. Irregular breathing following temporary decrease in hemorrhage. V. Decreased breathing due to depression of respiratory center. VI. Slow, shallow breathing. VII. Apnea. VIII. Asphyxial contractions of heart. IX. Asphyxial movements of respiration. Heart very slow and irregular.

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[37] Wiggers: Am. Jour Physiol., 1914, xxviii, 382. (See Figures 4 and 7.

SEQUENCE OF EVENTS DURING HEMORRHAGE

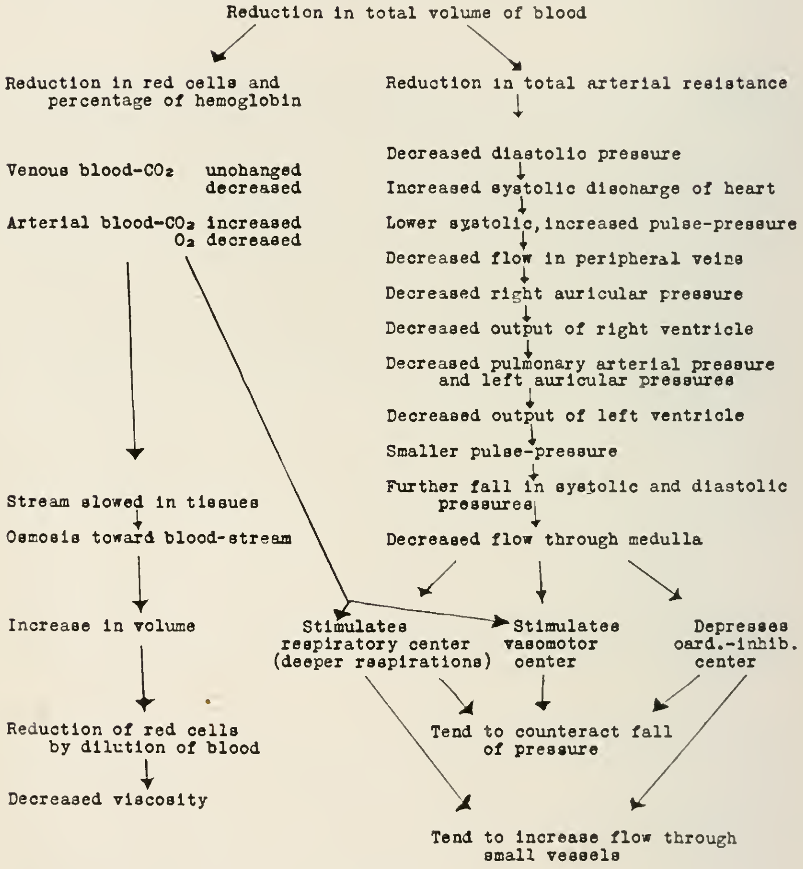
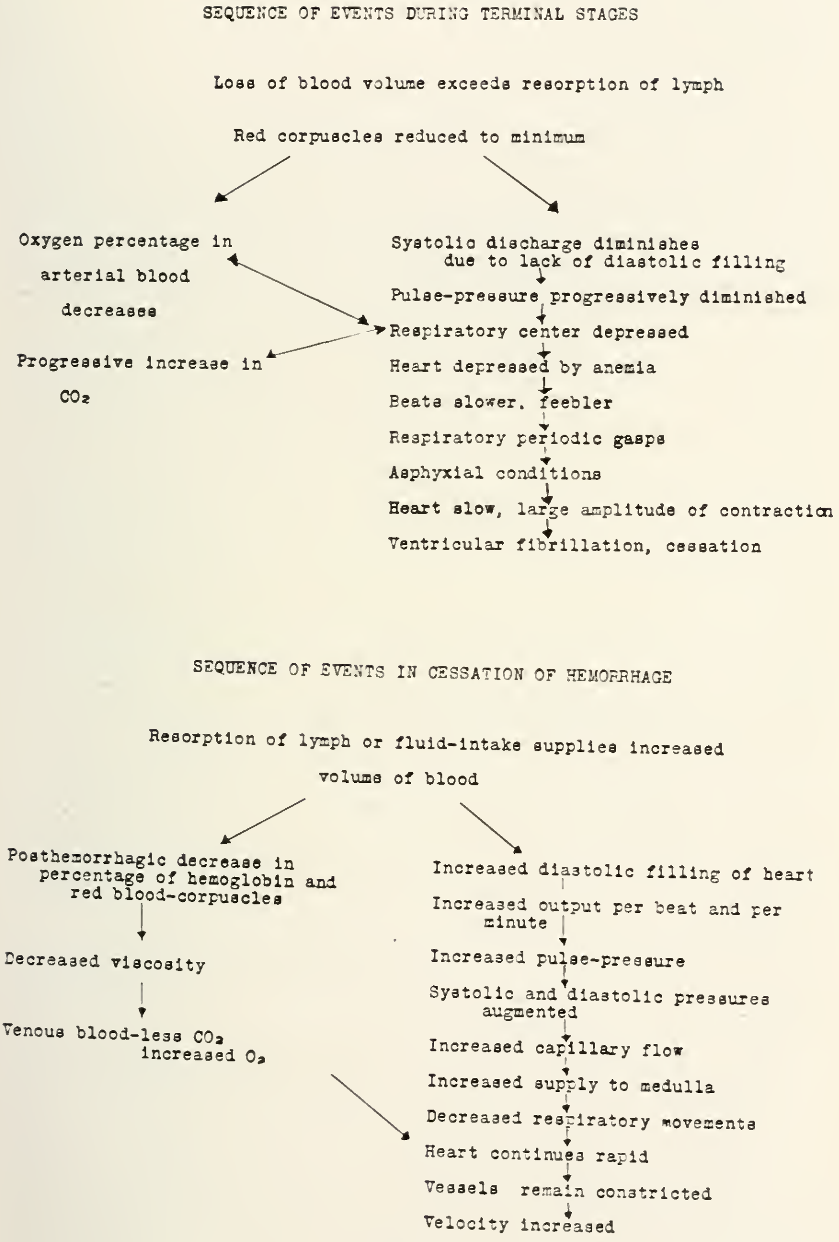

Reduction in total volume of blood
Reduction in red cells and percentage of hemoglobin
Venous blood-CO_2 unchanged
decreased
Arterial blood-CO_2 increased
O_2 decreased
Stream slowed in tissues
Osmosis toward blood-stream
Increase in volume
Reduction of red cells by dilution of blood
Decreased viscosity
Reduction in total arterial resistance
Decreased diastolic pressure
Increased systolic discharge of heart
Lower systolic, increased pulse-pressure
Decreased flow in peripheral veins
Decreased right auricular pressure
Decreased output of right ventricle
Decreased pulmonary arterial pressure and left auricular pressures
Decreased output of left ventricle
Smaller pulse-pressure
Further fall in systolic and diastolic pressures
Decreased flow through medulla
Stimulates respiratory center (deeper respirations)
Stimulates vasomotor center
Depresses card.-inhib. center
Tend to counteract fall of pressure
Tend to increase flow through small vessels

Figure 8.



SEQUENCE OF EVENTS DURING TERMINAL STAGES

Loss of blood volume exceeds resorption of lymph

Red corpuscles reduced to minimum

Oxygen percentage in arterial blood decreases

Progressive increase in CO_2

Systolic discharge diminishes due to lack of diastolic filling

Pulse-pressure progressively diminished

Respiratory center depressed

Heart depressed by anemia

Beats slower, feebler

Respiratory periodic gasps

Asphyxial conditions

Heart slow, large amplitude of contraction

Ventricular fibrillation, cessation

SEQUENCE OF EVENTS IN CESSATION OF HEMORRHAGE

Resorption of lymph or fluid-intake supplies increased volume of blood

Posthemorrhagic decrease in percentage of hemoglobin and red blood-corpuscles

Decreased viscosity

Venous blood-less CO_2 increased O_2

Increased diastolic filling of heart

Increased output per beat and per minute

Increased pulse-pressure

Systolic and diastolic pressures augmented

Increased capillary flow

Increased supply to medulla

Decreased respiratory movements

Heart continues rapid

Vessels remain constricted

Velocity increased

Figure 9.

THE BLOOD-PRESSURE IN PNEUMONIA *

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For decades clinicians have believed that many of the grave symptoms and a large number of the deaths in pneumonia were directly due to failure of the circulation. In 1889 Romberg[1] and his associates studied the problem and reached the conclusion that, while fatal circulatory failure did occur in rabbits inoculated with lethal doses of pneumococci, the failure was not of myocardial origin as had been supposed, but was invariably caused by the exhaustion of the vasomotor center in the bulb.

Since that time physicians have tried to combat the failure of the vasomotor mechanism in pneumonia by administering drugs which would augment the activity of the vasomotor center.

The results have been sufficiently confusing to make a re-investigation desirable. The present communication deals only with the blood-pressure in pneumonia. Other phases of the subject will appear in later papers.

If it be true that grave symptoms and death in pneumonia be caused by failure of the vasomotor apparatus, then it ought to be possible to divide cases of lobar pneumonia into two groups:

1. Those in which the vasomotor apparatus is intact.
2. Those in which the vasomotor apparatus is seriously injured.

In the first group the blood-pressure measurements ought to fall on a curve which does not diverge widely from that for normal individuals. In the second group the blood-pressure curve ought to be strikingly below the normal and to fall still lower as the gravity of the vasomotor disturbance increases. The blood-pressure curve ought to be the most reliable single measure of the state of the circulation in pneumonia, and a definite and exact indicator for our therapeutic efforts.

In 1911 Weigert[2] studied the blood-pressure in a variety of infectious diseases, including pneumonia, and reviewed the work of the

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* From the East Medical Service of the Massachusetts General Hospital.

† Aided by grant from the Council on Pharmacy and Therapeutics of the American Medical Association.

[1] Romberg, Pässler, Bruhns and Müller: Experimentelle Untersuchungen über die allgemeine Pathologie der Kreislaufstörung bei acuten Infectionskrankheiten, Arch. f. klin. Med., 1899, lxiv, 652.

[2] Weigert, K.: Ueber das Verhalten des Arteriellen Blutdrucks bei den acuten Infectionskrankheiten, Samml. klin. Vortr. (Volkmann's) Inn. Med., No. 138, p. 459.

earlier observers. He found that the statements of his predecessors were so contradictory that he could draw no conclusions from them. Among his own thirty-five cases there were six deaths. The pressures were not low in these cases. He separated the recoveries into several groups, that is: In six cases there was no effect on pressure. In nine cases the curve showed a gradual progressive fall from 25 to 35 mm. which reached its lowest point, on the average, on the ninth day after the crisis and was unaffected by the crisis. In three cases there were steep falls which bore no relation to the crisis. In the twelve remaining cases the curve showed a progressive rise. In several instances the pressure rose 40 mm. during the crisis. Weigert found that he could not explain these enormous variations on the basis of age, sex, general strength of patient, severity or length of the disease, the extent of dyspnea, the kind or the amount of stimulation. His general conclusion is that one can establish no rule for the blood-pressure curve in pneumonia and that, consequently, blood-pressure readings in pneumonia are of no prognostic value.

In contrast to this view is the statement of G. A. Gibson,[3] that "a pressure appreciably below the normal in pneumonia is invariably of evil omen and any considerable fall bodes disaster. When the arterial pressure, expressed in mm. Hg does not fall below the pulse-rate, expressed in beats per minute, the fact may be taken as of excellent augury, while the converse is equally true. From the work of the last few years in my own wards no fact is more certain than this." This is known as Gibson's rule.

Gordon[4] confirmed Gibson's statement on the basis of observations of fifteen cases. Unfortunately the data from which Gordon's conclusions are drawn are omitted from his paper.

The blood-pressure in fifteen cases of lobar pneumonia occurring on the East Medical Service of the Massachusetts General Hospital from March 1, 1913, to Dec. 1, 1913, have been studied. The measurements have, as a rule, been made in the middle of the morning and the middle of the afternoon on each patient from entrance until death, or, in the case of recovery, well into the convalescence. All cases in which there was any doubt about the diagnosis and all persons who had any form of chronic cardiovascular disease were excluded. Further than this, there was no selection.

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3. Gibson, G. A.: Some Lessons from the Study of the Arterial Pressure, Edinburgh Med. Jour., 1908, n. s., xxiii, 17.

4. That point at which the fourth tone phase passed into the fifth was called the diastolic pressure.

5. Gordon, G. A.: Some Remarks on the Value of Accurate Blood-Pressure Estimation, Edinburgh Med. Jour., n. s., iv, 31.

All measurements were made by one of us (G. R. M.). The apparatus employed consisted of a graduated U-tube containing mercury attached to a broad arm-band. All the readings were made by the auscultatory method. Not only the systolic has been measured, but also the diastolic[4] and pulse-pressure. It was hoped in this way to gain more information than could be obtained by studying merely the systolic pressure.

There were five deaths in the group of fifteen cases. The cases of recovery were roughly divided into two groups, mild and severe according to the general clinical impression they made, regardless of the blood-pressure.

Figure 1 presents a summary of the blood-pressure data. The abscissae represent blood-pressure in millimeters of mercury. The space between each two ordinates represents a day of twenty-four hours. The last day on the chart is the day of the crisis or the day of death as the case may be. The upper and lower unbroken lines are the average of the systolic and diastolic pressures in the fatal cases. The averages were calculated in each case by placing all the measurements obtained during the last day of life in the last column on the right of the chart; the measurements of the next to the last day of life in the next to last column of the chart and so on until we had reached the day of entrance. In this way composite data for similar periods of the disease are obtained. The individual measurements for the fatal cases are indicated by X. The heavy broken lines represent the averages of the systolic and diastolic pressures of the persons who recovered. In this group all the measurements obtained on the critical day were placed in the last column of the chart, and all measurements obtained on the day preceding the crisis in the next to last column of the chart. The individual measurements are indicated by dots and the outer limits of the field by the fine broken line.

It is apparent that the systolic pressure in the fatal cases was continuously above the systolic pressure of the persons who recovered. We are, consequently, unable to accept Gibson's dictum that "a pressure appreciably below the normal is invariably of evil omen." It will be noted that the diastolic pressure of the recoveries shows a slight but progressive fall while the systolic pressure remains very nearly level. This results in an increasing pulse-pressure as the crisis is approached. The fatal cases show a slight tendency in the opposite direction. But the difference is small. By consulting the accompanying table it will be seen that the average of the pulse-pressures on the day of death in the fatal cases is 6 mm. less than the average of the pulse-pressures of the recoveries on the critical day.

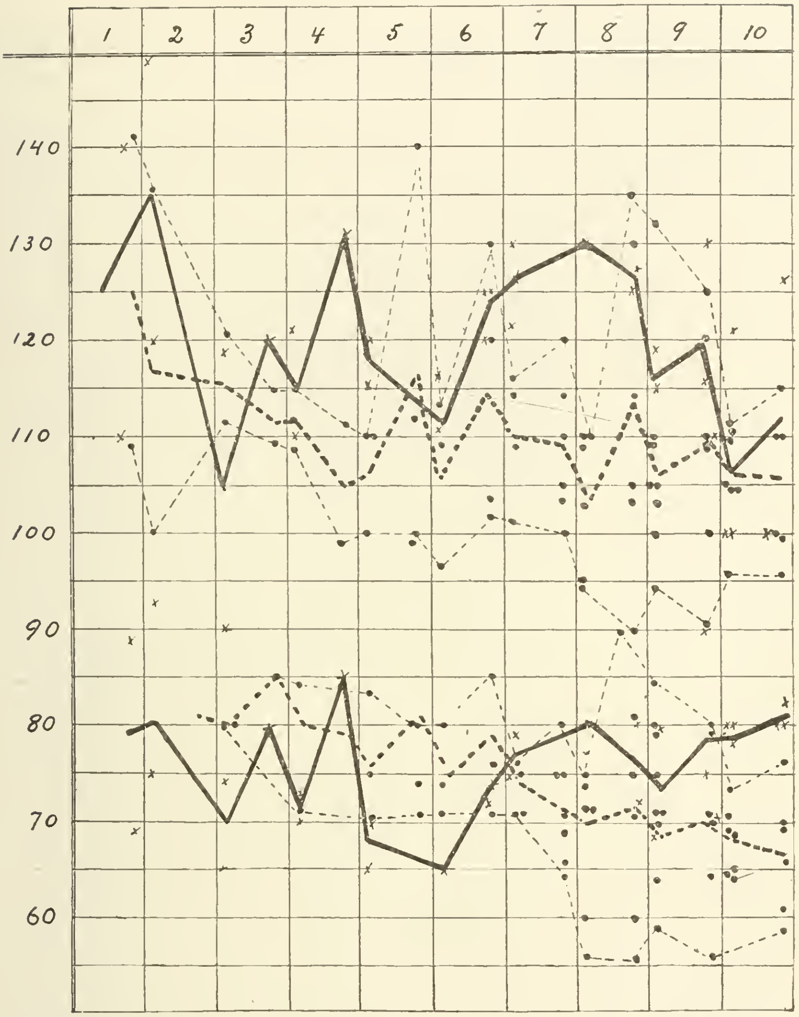

Fig. 1.—Presents a summary of the blood-pressure data from Massachusetts General Hospital cases. The abscissae represent blood-pressure in millimeters of mercury. The space between each two ordinates represents a day of twenty-four hours. The last day on the chart is the day of the crisis or the day of death as the case may be. The upper and lower unbroken lines are the average of the systolic and diastolic pressures in the fatal cases. The averages were calculated by placing all the measurements obtained during the last day of life in each case in the last column on the right of the chart; the measurements of the next to last day of life in the next to last column of the chart and so on until we had reached the day of entrance. In this way composite data for similar periods of the disease are obtained. The individual measurements for the fatal cases are indicated by X. The heavy broken lines represent the averages of the systolic and diastolic pressures of the persons who recovered. In this group all the measurements obtained on the critical day were placed in the last column of the chart. All measurements obtained on the day preceding the crisis were placed in the next to last column of the chart. The individual measurements are indicated by dots and the outer limits of the field by the fine broken line.

The table shows further that while the smallest pulse-pressures—20 mm.—occurred in two fatal cases, the largest pulse-pressure of the series was found also in a fatal case.

In Figure 2, two charts of temperature, pulse and respiration are reproduced to show that death may occur with a small or a large pulse-pressure, with a rising or a falling systolic pressure, with a systolic pressure above or below the pulse.

THE BLOOD-PRESSURE IN PNEUMONIA: READINGS ON DAY OF CRISIS OR DAY OF DEATH

Type	Systolic Pressure	Diastolic Pressure	Pulse-Pressure
Fatal............	120–125	70	50
Fatal............	125	80	45
Fatal............	100	80	20
Fatal............	100	80	20
Fatal............	105	70–75	30–35
Average.........	110	77	34
Severe...........	120	80	40
Severe...........	110–125	70–80	40–50
Severe...........	110	70	40
Average.........	116	75	42
Mild.............	100	70	30
Mild.............	110	70	40
Mild.............	110	80	30
Mild.............	105	65	40
Mild.............	100	60	40
Mild.............	115	70	45
Mild.............	102	63	40
Average.........	106	68	38

Gibson has stated that "when the arterial pressure, expressed in mm. Hg, does not fall below the pulse-rate expressed in beats per minute, the fact may be taken as of excellent augury, while the converse is equally true." An analysis of the cases fails to justify this statement. Among five fatal cases the systolic pressure fell above the pulse twice—an error of 40 per cent. Among ten recoveries the systolic pressure failed to fall above the pulse five times—an error of 50 per cent.

Owing to the great kindness of Dr. L. F. Barker and Dr. W. S. Thayer, we have had access to the records of cases of pneumonia treated at the Johns Hopkins Hospital. For purposes of study the last fourteen deaths and the last sixteen recoveries from lobar pneumonia have been selected. Cases of children, all cases in which the records were incomplete and also all in which there was any doubt about the diagnosis have been excluded. The pressure was measured in a routine way, two or more times daily by a number of persons; but, as a rule, the same person made all or nearly all the measurements on any single case. Systolic pressure only was recorded.

The course of the systolic pressure in the fatal cases has been compared with that of the persons who recovered, using the same method as that employed for making Figure 1. Figure 3 shows the two curves. It will be noted that the curves of systolic pressure in both Figure 1 and Figure 3 lead to the same conclusions, that the systolic pressure in fatal cases tends to be higher than it is in the recoveries.

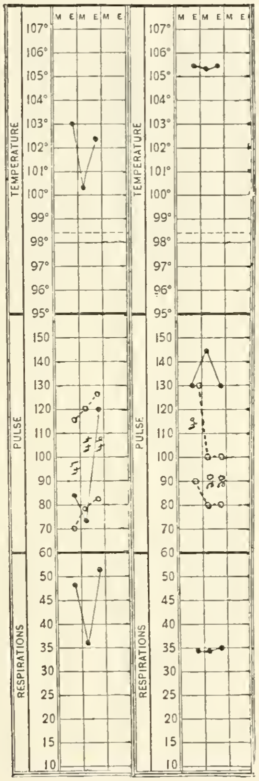

Fig. 2.—Charts of temperature, pulse and respiration in two fatal cases of pneumonia, showing that death may occur with a small or a large pulse-pressure and with a rising or falling systolic pressure, with a systolic pressure above or below the pulse. The lines of circles and dashes represent systolic and diastolic blood-pressure respectively.

The working of Gibson's rule in these Johns Hopkins cases is as follows:

Among 14 fatal cases, 6 followed the rule, 4 were doubtful, 4 did not follow the rule, that is, the rule held 6 times out of 14 (43 per cent.).

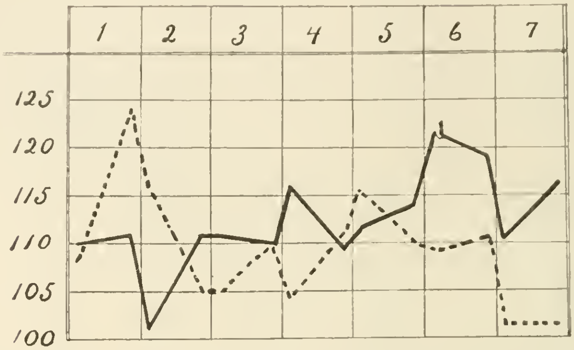

Fig. 3.—Curves of systolic pressure of fourteen fatal cases of pneumonia compared with sixteen recoveries taken from the records of the Johns Hopkins Hospital. Figure 3 was constructed in the same way as Figure 1.

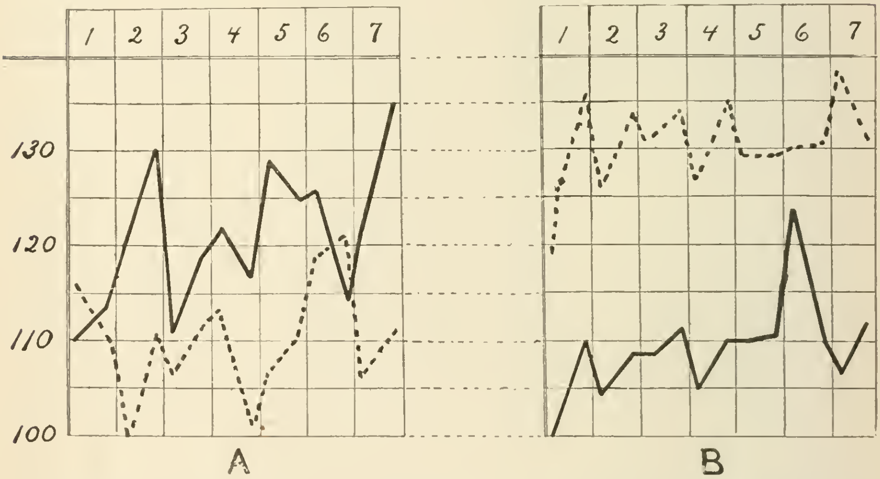

Fig. 4.—A: The solid line is the curve of systolic pressure in six fatal cases of pneumonia in which the systolic pressure was above the pulse. The broken line is the curve of systolic pressure in eight fatal cases in which the systolic pressure was below the pulse. B: The solid line represents the pulse rate in the same six fatal cases in which the systolic pressure is represented in A. The broken line is the pulse rate in eight fatal cases in which the systolic pressure is represented by the broken line in A.

Among 16 recoveries 6 followed the rule, 2 were doubtful, 8 did not follow the rule, that is, the rule held 6 times out of 16 (37 per cent.).

In both groups the rule held only in a minority of the cases and was definitely wrong 40 per cent of the time.

Lastly all the fatal cases (those of the Johns Hopkins Hospital and those of the Massachusetts General Hospital) have been divided into two groups with regard to the relation of the systolic pressure to the pulse. The first group includes the cases in which the systolic pressure expressed in millimeters of mercury fell above the pulse expressed in beats per minute. The second group includes the cases in which the systolic pressure fell below the pulse. In the first group there are eight cases, in the second, eight cases.

Figure 4 shows the relation of the pressures and the pulses in the two groups. It will be seen that the pressure curves fall very much closer together than the pulse curves. This immediately suggests that the rate of the pulse and not the level of the pressure, is the chief factor in deciding whether the pressure will fall above or below the pulse. That such is actually the fact can easily be determined by comparing the averages of all the measurements used in constructing each curve. By so doing, it is found that the average of the systolic pressures for the first group is 121, for the second group, 109, a difference of 11; whereas the averages for the pulses in the first group and second group are respectively 131 and 109, a difference of 22. The rate of the pulse is exactly twice as important as the height of the systolic pressure in determining whether the latter shall fall above the former or vice versa.

SUMMARY

1. The blood-pressure curve does not suggest that there is a failure of the vasomotor center in pneumonia.
2. Low systolic pressures are not "invariably of evil omen."
3. The systolic pressure in these fatal cases tends to be higher than it is in the recoveries.
4. Blood-pressure measurements in pneumonia cannot be used as a basis for treatment.
5. Prognostic inferences based on the relation of the level of the systolic pressure curve to the pulse curve (Gibson's rule) are wrong more often than they are right in this series.
6. The rate of the pulse and not the level of the pressure is the chief factor in deciding whether the pressure will fall above or below the pulse.
7. The pulse-pressure data are insufficient in number to justify conclusions.

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PNEUMOCOCCUS INFECTION AND LOBAR PNEUMONIA *

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Pneumonia, in many respects, certainly as a cause of death, is the most important infectious disease with which we have to deal. The symptomatic treatment is difficult and of doubtful utility; there is no well-established form of specific therapy. Nevertheless, up to within a very recent time, the investigation of the real nature of the process has been slight and fragmentary.

While the association of certain kinds of bacteria with this disease has been well established, much obscurity exists with regard to the mode of infection, the relation of the bacteria to the lesions and symptoms, the nature of recovery and, above all, with regard to the possibility of prevention or of cure. It has been in the hope of helping to shed some light on these problems that my associates and I have been making some clinical and experimental studies. It will be impossible to review in this paper all the work that has been done by others, and I shall have to content myself with presenting certain points of view which have been suggested mainly by our own work at the Rockefeller Institute.

Acute lobar pneumonia seems the best characterized of the acute lung affections. It has such a clear-cut, clinical course that it is now generally considered a distinct clinical entity, and no more to be regarded simply as an infection of the lung than typhoid fever is to be considered an infection of the intestine. This, however, is a clinical point of view and it is possible that the same kinds of reaction occur in other localized pneumococcus infections as are present when the main seat of infection is the lung.

In a very large percentage of patients suffering from inflammation of the lung of the lobar type, pneumococci are present in the lesion. In isolated instances, other organisms, as *Bacillus influenzae* or *B. pneumoniae,* are found in pure culture. It has not been our purpose to consider these latter cases, but our attention has been given entirely to the group of cases in which *Diplococcus pneumoniae* is present and is apparently the etiological agent. As is well known, diplococci, which

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* From the Hospital of the Rockefeller Institute.

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at present cannot be differentiated from the pneumococcus, may be present in pneumonia of the lobular tube (in which the clinical course is quite distinct from that in lobar pneumonia) ; they may also be present in other purely localized lesions in the body, entirely unassociated with any affection of the lung, and they may even be the organisms concerned in certain cases of septicemia in man without any local lesions whatever. Moreover, organisms with identical characteristics, so far as yet determined, are found with so great frequency living on the mucous membranes of the mouth and throat of perfectly healthy individuals that they may be considered normal inhabitants of the mouth and throat cavities. In the face of such facts as these, how can it be maintained that *Diplococcus pneumoniae* is the primary cause of such a well-characterized acute infectious disease as acute lobar pneumonia? In view of the present general consensus of opinion that this theory is true, one is indeed rash even to suggest the possibility that there may be another agent concerned. On the other hand, it is important that such a possibility should not be overlooked. Even though it should be shown, however, that pneumococci do not play the primary etiologic rôle in the natural infection, their association with the lesion and their frequent invasion of the blood render it evident that they play an important part in the process and probably the most important part in the outcome, just as do streptococci in certain diseases, such as small-pox and scarlet fever, of which it is generally believed that the natural infection is due to specific etiologic agents.

Up to within a relatively short time, the most important link in the chain of evidence that pneumococci cause pneumonia, namely the reproduction of the disease in animals, was lacking. Most important studies dealing with the experimental production of acute lobar pneumonia were published in 1904 by Wadsworth.[1] By carefully balancing the general resistance of the animal with the virulence of the race of pneumococci employed and by injecting the organisms intratracheally, he was able, in a series of rabbits, to induce a diffuse exudative pneumonia like the acute lobar pneumonia seen in man.

More recently Lamar and Meltzer,[2] and Wollstein and Meltzer[3] have succeeded in regularly producing a diffuse pneumonia of the lobar type in dogs, by injecting from 10 to 15 c.c. of the fluid culture directly into one bronchus through a rubber tube passed through the trachea, following the injection of the fluid by air blown through the tube, so as to force the infectious material into the finer ramifications of the bronchi. The pneumonia produced in dogs runs a more rapid course, resolution occurs earlier—in three or four days—and the mortality is much less than in pneumonia in man.

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[1] Wadsworth: Am. Jour. Med. Sc., 1904, cxxvii, 851.
[2] Lamar and Meltzer: Jour. Exper. Med., 1912, xv, 133.
[3] Wollstein and Meltzer: Jour. Exper. Med., 1913, xvii, 353, 424.

Using a similar technic, these investigators have produced diffuse lesions in the lungs of dogs with other micro-organisms. When streptococci are injected, the lesions tend to resemble more closely those seen in bronchopneumonia in man.[4] The observers lay stress on the greater tendency in this case to a leukocytic infiltration of the lung framework, and to a much-lessened formation of fibrin. These differences between the pneumonia produced by the injection of streptococci and that following the injection of pneumococci they ascribe to inherent differences in the nature of the micro-organisms concerned, and not to relative differences in virulence. By a similar method of intratracheal injection, Winternitz and Hirschfelder[5] have succeeded in producing pneumonia of a lobar type in rabbits. In these experiments also, large amounts of the culture material (4 or 5 c.c.) were injected.

From experiments which I have carried on, using the same method, it is evident that successful results in rabbits depend somewhat on the race of organisms employed. If the organisms have very slight virulence, the animals may recover without lung lesions; if they are too virulent, a septicemia quickly results and at necropsy only congestion and edema of the lungs are present.

As Wadsworth showed, the lung consolidation is probably a manifestation of the resistance of the animal to the spread of the infection. The occurrence of the diffuse lung lesion is undoubtedly dependent on the same factors which are concerned in the differences in local reaction to the injection of virulent pneumococci in different races of animals. As is well known, mice and rabbits are very susceptible to pneumococcus infection, however induced, guinea-pigs and dogs less and man possibly still less. The result of a subcutaneous injection of virulent pneumococci into a rabbit differs markedly from that seen when a similar injection is made into a guinea-pig. In the former there is very little local reaction; a rapid general invasion of the organisms takes place, and the animal dies quickly from a marked septicemia. In the latter a marked infiltration with much fibrin formation and a slowly progressive invasion of the subcutaneous tissues occur, while there is little or no general infection. That the time element plays a rôle in the formation of fibrin is well seen in the peritonitis induced in such susceptible animals as the mouse and rabbit. If an intraperitoneal injection is made of virulent pneumococci, death occurs within twenty-four hours, and in the peritoneum there is seen only

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4. Wollstein and Meltzer: Jour. Exper. Med., 1913, xviii, 548.
5. Winternitz and Hirschfelder: Jour. Exper. Med., 1912, xvii, 657.

a marked congestion, possibly hemorrhages, a serous exudation and usually no fibrin. If, however, the culture is less virulent and the animal lives forty-eight hours or more, there is usually considerable fibrin over the liver, and flakes of fibrin are seen throughout the cavity. The amount of fibrin increases in ratio with the length of time during which the animal is able to resist the infection.

The experiments of Wollstein and Meltzer,[4] however, tending to show that the peculiar property of stimulating the production of fibrin is possessed to a greater degree by pneumococci, whatever their virulence, than by streptococci, is of very great significance. It is difficult to understand, however, just why this property should be a factor in the production of pneumonia of a lobar rather than of a lobular type. That this peculiar property should not be the only one concerned is made evident from the fact that the pneumococcus is the organism most frequently concerned in lobular pneumonia in children. Dochez[6] has shown that, during the acute stages of lobar pneumonia, there is an increase of fibrinogen in the blood. Nevertheless, the coagulation time of the blood is delayed, owing probably to an increase in antithrombin as well. We know of no observations which show whether or not so great an amount of fibrinogen exists in the blood in prolonged streptococcus infections in man as is present in pneumonia.

As the virulence of the race employed is of importance as regards the production of the local reaction, so also is the number of microorganisms injected, as Kline and Winternitz[7] have shown. If the number of organisms is too small, no pneumonia results. It is well known that even in very susceptible animals a considerable number of virulent organisms is usually necessary to produce infection. Little attention has been given to the question why, when a considerable number of organisms are injected together, multiplication occurs and infection results, whereas if only a few organisms be injected, they are unable to multiply. Is it because in a culture of organisms certain ones are more resistant than others to the harmful influences, or is it simply accidental that, when a large number of organisms are injected, a few have a possible chance to escape?

Gillespie[8] has carried on some experiments with pneumococci which have a bearing on this problem. We, as well as others, have long recognized that in starting a culture of pneumococci in a large amount of bouillon, a liter for instance, a much larger inoculation is necessary in order to obtain growth than if the inoculation be made into 10 c.c., or if the culture be made on a solid medium. In the latter instance,

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6. Dochez: Jour. Exper. Med., 1912, xvi, 693.
7. Kline and Winternitz: Jour. Exper. Med., 1913, xviii, 50.
8. Gillespie: Jour. Exper. Med., 1913, xviii, 584.

one organism will usually produce a colony. By making the inoculations on filter-paper kept constantly wet by bouillon, it was shown that growth would occur with the inoculation of as small numbers of organisms as are required on agar, and with much smaller numbers than are required to inoculate the bouillon. The differences in growth, therefore, are not dependent on differences in composition of the medium, and further experiments have shown that they are not due to differences in oxygen supply. It seems probable that for growth to occur, the bacterium must produce changes in the medium immediately surrounding it, and that when the opportunities for diffusion are great, such local changes cannot be kept sufficiently constant unless there be a considerable number of organisms in proximity. If this be the true explanation, it may have an important bearing on infection, not only with pneumococcus but with other micro-organisms as well. The presence of mucus in the smaller bronchi, for instance, might in this way favor the multiplication of micro-organisms and so favor infection. That very large numbers of bacteria are inoculated in the experiments of Meltzer and Winternitz probably explains to some degree why their experiments have been successful, where others have failed. A second factor in the success of this technic probably lies in the fact that considerable amounts of fluid are injected and this is blown into the terminal bronchioles. Meltzer has made the interesting suggestion that by this process the bronchioles are occluded and that in this way closed cavities are formed. It is generally recognized that as long as bacteria grow on exposed surfaces, they do no harm. It is only when the growth occurs in confined spaces that harmful results supervene.

While these experiments on the production of pneumonia in animals are of great value in showing that lesions resembling acute lobar pneumonia in man may be caused by pneumococci, they do not directly offer an explanation of the natural infection in man. It is hardly likely that in man an overwhelming infection ever occurs with numbers of bacteria so large as those used in the experiments in dogs. The usual recourse in this dilemma is to assume that the organisms concerned in natural infection have an increased virulence, or that the resistance of the host is lowered. By virulence in micro-organisms is usually meant adaptation to growth in the tissues of the host. Since in pneumonia the organisms are cultivated from the lungs and at times from the blood, we know that they have virulence for man. We have no way of determining, however, whether or not all pneumococci growing in the mouths of healthy individuals are also virulent for man. The attempts to demonstrate conclusively that pneumococci isolated from cases of pneumonia in man are regularly of increased virulence for animals have not been successful. We have found that most of the pneumococci isolated from the blood of pneumonia patients have relatively high virulence for susceptible animals, yet while certain of these cultures, when freshly isolated, are of such virulence that 0.000001 c.c. of a bouillon culture will kill a mouse, we have also obtained cultures that required 0.5 c.c. to kill. The virulence of the race, therefore, does not seem to be the only deciding factor in the question why infection occurs, even though, as will be shown, it may be of considerable importance as regards the final outcome. It must be stated, however, that it is not certain that virulence for animals is identical with virulence for man. Usually high virulence of a given race of pneumococci for one susceptible animal, as the rabbit, indicates high virulence for another, as the mouse. Ungermann, however, has described a typical pneumococcus having high virulence for rabbits, but its virulence for mice, which was originally present, was lost. We have studied a race of pneumococci originally very virulent for mice, which, after passage through guinea-pigs, increased its virulence for these animals, but became almost avirulent for mice and rabbits.

Against the view that pneumonia arises when organisms of increased virulence reach the lung is the fact that pneumonia rarely occurs in epidemics and is very slightly contagious. There are now a number of epidemic outbreaks reported, but it must be admitted that these are of rare occurrence, and all hospital experience is against contagion as a great factor in the spread of the disease. On the other hand, the fact that in certain times and places pneumonia occurs with greatly increased severity and frequency suggests that at these times the pneumococci concerned may have acquired increased virulence. When the French attempted to build the Panama Canal, the incidence and mortality of pneumonia interfered as seriously with the work as did the occurrence of malaria and yellow fever. Even during the first years of the American occupation of Panama, the mortality from pneumonia was enormous. For its decrease there seems no adequate explanation. In South Africa, deaths from lobar pneumonia among the coolies constitutes a serious menace to the continued working of the mines.

Little is known concerning diminished general resistance on the part of man to pneumococcus infection and its importance in natural infection of this disease. Clinical studies have quite conclusively demonstrated that the habitual use of alcohol increases susceptibility to infection or, at any rate, renders the subject less resistant when infection has once occurred. That exposure to cold, or especially sudden changes in temperature and chilling, play a part in infection can hardly be doubted, and there is some experimental evidence to show that animals suddenly chilled are more susceptible to infection with pneumococci than others.

The view that local changes in the lung are of importance for the occurrence of infection is interesting and suggestive. In most cases of lobar pneumonia the primary seat of infection is probably in the lung. Various writers have attempted to show that infection occurs through the blood-stream, but the evidence is not conclusive. Other localized pneumococcus infections in internal organs or cavities usually occur by extension, though this is not always demonstrable. The possibility that infection through the blood may occur in certain instances cannot be excluded. Pneumonia, as a part of a general infection, however, is generally of a lobular type.

Much has been said lately about the adaptation of certain organisms for certain tissues. In many cases, however, this adaptation is more apparent than real, and the mode and degree of infection play the larger rôle in localization.

In about 50 per cent. of the cases of pneumonia, a history of preceding coryza and cough may be obtained. In these cases it is possible that there occurs a downward infection along the mucous membrane of the bronchi. The extension of the lesion through the lung from one lobe to another apparently takes place through the bronchi, as the study of large sections through lobes with beginning involvement shows. In the remaining half of the cases, however, the onset is sharp and sudden without history of bronchial involvement. Even in these cases some local change probably precedes the real infection. It is well known that thoracic trauma is frequently followed by pneumonia. The idea of Meltzer that infection may be facilitated by closure of the smaller bronchioles is most suggestive. It is possible that cold or chilling may stimulate the mucous glands so that the increase of mucus may produce favorable conditions for the growth of pneumococci, which are so frequently present in the upper respiratory tract.

That the lung is the chief seat of the disease, then, is probably due to the fact that infection occurs here, and that a local lesion results and not a general infection (at least not until late in the disease), is probably due to the fact that man is highly resistant to infection with pneumococci and that the anatomical conditions here permit of an extensive inflammatory reaction which opposes the spread of the infection.

But why does infection occur at all? Why does a person contract pneumonia? There is still considerable obscurity in regard to this phase of infection, not only in pneumonia, but also in many other infectious diseases of which the etiology is well known. The obscurity and difference of opinion in regard to tuberculosis is well known. Even our views with regard to infection in diphtheria have been disturbed by new observations. We all thought the transference of the infectious agent from the infected to the healthy throat was all that was necessary for infection in this disease. The observations of Moss, Guthrie and Gelien,[9] however, that in Baltimore there are four times as many carriers of virulent diphtheria bacilli as there are cases of the disease, are most disturbing. So far as can be determined, the bacilli from carriers differ in no way from the bacilli from patients with the disease. Moreover, the incidence of the disease seems to bear no relation to these carriers. The problem of mode of infection is thus in a minor degree analogous to that in pneumonia, in which practically the entire population represent carriers of infection.

To explain the nature of infection we may say: First, there is a possibility that in pneumonia, as in diphtheria, the organisms causing infection differ inherently from those in normal throats, especially as regards adaptation or virulence for men. Definite evidence in favor of this would be most important, but at present there is none. Second, it is possible that the general resistance of patients to pneumococci is lowered, so that organisms, formerly living as harmless parasites, now invade the tissues and induce reaction. For this also we have no definite evidence. Third, the study of artificial infection in animals, as well as the course of the disease in man, suggests most strongly that local changes in the respiratory tract may precede the infection with pneumococci. Whether these are due to a primary infectious agent or to changes in the tissues due to other factors cannot be decided definitely at present. Finally, it is possible that infection depends on a combination of factors, virulence of organisms and general and local resistance each playing a part. Further knowledge along these lines is absolutely essential for prevention of this disease. To formulate rules or regulations for prevention at present seems useless, except as an experiment.

THE NATURE OF THE INTOXICATION

Whatever be the mode of onset in pneumonia, the production of the local changes in the lungs, as well as the general systemic manifestations of the disease, seems to be in some way related to the growth of pneumococci in the body. When micro-organisms grow within other multicellular organisms acting as host, the effects on the host are of two kinds: First, there is a local reaction, in which the bacteria are present in considerable numbers, as at the point of infection. Here are induced the changes spoken of as inflammation. In addition to this, however, there is practically always a reaction throughout the entire body, even when the local reaction is very mild and evanescent. These general reactions are evidenced, not only by fever and nervous disturbances on the part of the host, but even in their absence by such effects as changes in the blood, especially the leukocytes, which indicate certain effects on the blood-forming tissues.

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[9] Moss, Guthrie and Gelien: Tr. Fifteenth Internat. Cong. on Hyg. and Demog., Washington, 1912, iv, 156.

In most cases, the exact manner in which micro-organisms stimulate the tissues in which they are growing to a reaction which is called inflammatory, is still obscure. Since, however, identical reactions may be produced with non-living chemical substances, it is generally assumed that in the case of bacteria as well, non-living chemical substances are formed as a result of the bacterial growth, which substances are in themselves harmful. Whatever may be the exact relationship of the organisms to the local lesion, it is necessary to assume that the general manifestations of infection, and especially the effects on tissues far distant from the local lesion, are the result of soluble toxic substances which circulate in the blood or lymph. Since general manifestations similar to those in pneumonia are seen in other pneumococcus infections and even in general infections in animals without local lesions, it does not seem probable that these effects in pneumonia are due to disturbances in respiration associated directly with the lung lesions. It is possible, of course, that in pneumonia the general manifestations and effects on distant tissues and organs are due to the action *in situ* of bacteria which have gained access to the circulation and have been carried to these distant parts.

Very numerous observations have been made on the occurrence of bacteria in the general circulation in acute lobar pneumonia. During the past years blood-cultures have been made on most of the cases of pneumonia coming under my observation, and the results have not led me to change the conclusions arrived at ten years ago from the study of a series of cases, namely, that pneumococci are usually found in the blood only in the more severe cases, and the presence of the pneumococcus in the blood is of ill omen. It is possible that in all cases of pneumonia an occasional bacterium may be carried into the circulation, but the demonstration of this is difficult. That this may occur, however, is not of prime importance, for the occurrence of an occasional organism could hardly explain the great degree of effect in distant tissues, as manifested by the general symptoms which we call intoxication.

The attempts to discover something of the nature of this circulating poison have been attended with much difficulty. It would seem that a more accurate knowledge of the metabolic disturbances in pneumonia might give a clue as to the nature of the intoxication. A series of studies with this object in view was undertaken.

Of late years attention has been drawn to the occurrence of functional disturbances, especially in infants, due to derangements in salt metabolism. It appeared of interest to learn whether or not specific changes in inorganic metabolism may be induced by pneumococci, which could account, in part at least, for the symptoms induced. The most striking disturbance in pneumonia is known to be the retention of chlorids, which is frequently almost complete during the acute course of the disease Retention of chlorids to a lesser degree is known to occur in other infections, but Rowntree[10] has shown that this retention does not occur in influenzal pneumonia to nearly so marked a degree as it does in pneumococcus pneumonia. Medigreceanu[11] has carried on a series of studies of pneumonia in dogs and Peabody[12] has studied the question in cases in man. Peabody has shown that there is a retention not only of chlorin, but also of sodium and calcium while there is no retention of potassium and magnesium, but may be a loss. Further studies indicate that the retained substances are not stored in any one place, but are spread diffusely throughout the tissues.

It is not believed that these changes are specific for pneumonia, for they probably occur in other infections. They are most striking in pneumonia, since the changes between the febrile and afebrile state occur with such suddenness. It is not likely that these changes in themselves are responsible for any of the symptoms of the disease, but in view of the striking effects which have been induced by Meltzer by changing the balance in the inorganic salts in the body, this possibility must be borne in mind. We have no knowledge of the reason for these changes in pneumococcus infection.

Pneumococci are known to produce acid readily, even, as shown by Hiss, in albuminous mediums, containing no demonstrable sugar. It has therefore been suggested that the symptoms in pneumonia are the manifestations of an acidosis. Hamburger[13] has attempted to explain the chlorin retention on the basis of a febrile acidosis. The studies of Peabody,[14] however, have shown that the curves of chlorin retention and of ammonia excretion, which is generally considered the best indicator of acidosis, do not necessarily run parallel. The studies of inorganic metabolism have therefore given no conclusive insight into the nature of the intoxication.

In order, if possible, to obtain some knowledge regarding this problem by another method, Peabody has made studies of the gas exchange

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10. Rowntree: Bull. Johns Hopkins Hosp., 1908, xix, 367.
11. Medigreceanu: Jour. Exper. Med., 1911, xiv, 289.
12. Peabody: Jour. Exper. Med., 1913, xvii, 71.
13. Hamburger, H. J.: Osmotischer, Druck und Ionenlehre, Berlin, 1912.
14. Peabody: Jour. Exper. Med., 1912, xvi, 701.

in the blood in pneumonia. He has found that in this disease the carbon dioxid in the venous blood is quite regularly low, in spite of the disturbances in gas exchange in the lung. At the same time there occurs an increase in the ammonia nitrogen in the urine, and the curves run somewhat parallel. These changes, which are indicative of increased acid formation, nevertheless correspond to changes that have been known to occur during fever and infection due to other causes, and are no indication of specific changes occurring in this disease. The carbon dioxid content of the blood does not bear a definite relationship to the severity of the disease, except that it is lowest in the most severe cases and in the terminal stages.

On the other hand, the study of the oxygen-content of the blood has revealed some interesting changes. Studies of the peripheral venous blood showed in certain cases a diminution in the oxygen-content of the venous blood. In studying the blood in one such case, it was found that the blood would not take up a normal amount of oxygen, and this in spite of the fact that the hemoglobin content was normal. In a careful study of such blood by Butterfield and Peabody[15] it was found that this phenomenon was due to the formation of methemoglobin. This change also occurs regularly in the blood of rabbits[16] infected with the pneumococcus and has no relation to the lung lesion. It also occurs when the bacteria are grown in blood-containing mediums.

Usually the change into methemoglobin in the animal body does not go so far that the methemoglobin can be distinguished spectroscopically. In the test-tube, however, especially when hemoglobin in solution is added to the culture, practically all the hemoglobin may be changed into methemoglobin. That this reaction is not simply due to the action of acids formed by the pneumococcus is shown by the fact that for the production of methemoglobin far more acid is required than could be present in the body, and, moreover, that it may occur in cultures or filtrates that are alkaline in reaction. It is therefore evident that this change is due to the action of a poison formed by the pneumococci.

Peabody[17] further made a study of the blood in twenty-five cases of pneumonia to determine the frequency of the occurrence of this phenomenon and the time of its appearance. Of the cases which ended in recovery, in only one was there any indication of a diminution of the oxygen-absorbing power of the hemoglobin. In all of the ten cases ending fatally, there occurred a progressive loss in the oxygen content of the blood and in the oxygen-combining power of the hemoglobin, and from the previous studies it is certain that these changes are due to the formation of methemoglobin. In nine of the ten cases the blood-cultures were positive.

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[15] Butterfield and Peabody: Jour. Exper. Med., 1913, xvii, 587.

[16] Peabody: Jour. Exper. Med., 1913, xviii, 1.

[17] Peabody: Jour. Exper. Med., 1913, xviii, 7.

That these changes play a part in the fatal termination can hardly be doubted. The terminal symptoms of the disease may be accounted for by deficient oxidation. It is not likely, however, that these changes in the blood are in themselves the only factor in accounting for the fatal result; but they represent one of the factors, and are an indication of the intoxication which is the result of the growth of pneumococci in the body.

A second effect of the pneumococcus intoxication has been demonstrated by Medigreceanu† by estimating the amount of oxydase in the organs of animals dying from pneumococcus septicemia, as compared with the organs of normal rabbits. In these studies Medigreceanu employed the method of Röhman and Spitzer, which is based on the property of tissues of oxidizing a mixture of naphthol and paraphenylendiamin into phenol. By comparing the tissues of normal animals with those previously infected with pneumococci, it has been found that this oxydase is generally diminished in the latter animals. By proper controls it has been possible to show that this change is due, not to the presence of pneumococci in the tests, but to some change which results in the tissue from the infection. Another effect of the action of the toxin on tissue function is thus made evident. It is therefore probable that, in addition to the lessened supply of oxygen by the blood due to the formation of methemoglobin, there is also a lessened power of the tissues to carry on the proper oxidation function.

Finally, in order to obtain evidence of the presence of a poison, studies were made by Medigreceanu[18] to determine whether or not there was an increased output of substances known to have the property of neutralizing poisons arising in the body. Such a substance is glycuronic acid, and it was found that during the acute stages of pneumonia in man, in almost all cases, there is a definite increase in the output of this substance.

All these studies clearly indicate the activity in pneumonia of a circulating poison; but the direct demonstration of the presence of this toxic substance in the animal is more difficult. To this end the following experiments were performed. Each one of a series of rabbits was inoculated with an overwhelming dose of pneumococci. Then, just as death was imminent in from five to eight hours, the animal was bled to death, and as quickly as possible the blood was defibrinated, the serum passed through a Berkefeld filter to remove the bacteria and the filtrate injected intravenously into a normal rabbit. To our surprise and disappointment, the animals did not die, nor in a second series of rabbits treated in this way were we able to detect any minor harmful effects of such injections.

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† Medigreceanu: Jour. Exper. Med., 1914, xix, 309.

[18] Medigreceanu: Jour. Exper. Med., 1913, xviii, 259.

When one considers the conditions in pneumococcus infection it is not surprising that there is great difficulty in demonstrating the presence of toxin in the animal, or even of demonstrating the production of toxin by the pneumococcus *in vitro*. The infectious diseases in which active specific toxins have been well demonstrated *in vitro* are diphtheria and tetanus. In these diseases, however, the conditions are unusual. Here a moderate number of organisms growing in the local lesion produce sufficient poison to bring about the most profound intoxication, and it is not surprising that the poison may readily be demonstrated *in vivo* and *in vitro*. In pneumococcus infection, the conditions are different. Even in general infection in the highly susceptible mouse or rabbit, the number of organisms growing in the body is enormous before the animal finally succumbs.

In the severe and fatal cases in man the blood may contain as many as 65,000 organisms per cubic centimeter; and when it is conceived that these are throughout all the body-fluids and the tissues, it is evident what immense numbers of bacteria are responsible for the intoxication and fatal outcome. In man, even when there is no marked invasion of the blood, the number of micro-organisms in the lung must be very large. It is probable that during the course of the disease bacteria are all the time undergoing degeneration, so that from the beginning to the end, large numbers of bacteria have been present. Also the amount of toxin present at any one time may be very small, yet when acting on tissue-cells for six or seven days may produce marked effects.

The suggestion has been made that in pneumonia the symptoms are due to the absorption of products of digestion of the pathological exudate in the lung. It has been well established by various observers that, during the parenteral digestion of protein, substances are formed which may induce fever and symptoms of intoxication. Similar symptoms may be induced by the injection of peptone and other products of protein digestion, into the circulation of animals. Most of the work that has been done in the production of fever by means of protein, however, has been carried out with foreign protein and not with the protein of the host. Moreover, at the time the resolution is probably greatest, that is, following the crisis, such symptoms are not present, although in most cases considerable amounts of the digested exudate are being absorbed. It is hardly likely that the specific and characteristic symptoms of pneumonia can be due merely to the absorption of the products of digestion in the local lesions.

Following the discovery of serum anaphylaxis in guinea-pigs and its relation to protein intoxication in man, numerous efforts have been made to bring this into relation to the toxic effects seen in acute infectious disease. Friedberger has shown that if bacteria be treated with immune serum and then with complement, the supernatant fluid, after removal of the bacteria by centrifugalization, will be toxic when injected into a guinea-pig, the animal dying within a few minutes with symptoms identical with those seen in anaphylactic shock. He has called the toxic substance produced outside the body in the manner stated "anaphylatoxin," and believes that it is identical with the substance formed within the body which produces the symptoms following the second injection of protein. He thinks that this experiment may offer the explanation for all bacterial intoxications. According to his theory in the period of incubation, during which the bacteria are already present but produce no symptoms, antibodies are being formed, and when these are present in sufficient numbers, the bacterial bodies begin to be split up and the substances so formed produce, not the acute symptoms which are speedily followed by death, because the bacteria are not present in sufficient numbers, but milder symptoms —fever, etc., a kind of chronic anaphylaxis. This can hardly explain, however, what occurs in pneumonia, in which all the evidence seems opposed to a long incubation period, the onset of the symptoms being sudden and apparently the immediate result of the infection.

The production of the so-called "anaphylatoxin" from pneumococci may readily be done, as we and also Neufeld and Dold have shown. Neufeld and Dold,[19] moreover, have shown that similar toxic substances may be obtained from bacteria by simple extraction in salt solution containing lecithin. Rosenow[20] then showed that if pneumococci are merely placed in salt solution for forty-eight hours at 37 C. (98.6 F.), the extract so formed is toxic, and on injection intravenously into guinea-pigs, acute symptoms and speedy death, like those seen in serum anaphylaxis, result. We have studied the effect of the injection of extracts obtained by autolysis in a very large number of guinea-pigs,[21] and, in our experience, while occasionally sudden death is produced, this does not occur with great regularity.

Since the salt solution extracts of pneumococci did not show as high toxicity as was anticipated, it was held possible that in the peritoneal cavity of an animal the solution of the bacteria might go on at a more rapid rate, from which cavity solutions might be obtained of greater and more constant toxicity. Guinea-pigs were therefore inoculated intraperitoneally with large doses of pneumococci. As soon as possible after death, the peritoneal cavity was washed out with salt solution. The cells and bacteria were then removed from this solution by centrifugalization and the supernatant fluid was used for intravenous injection into healthy guinea-pigs. Of eleven animals so treated, eight showed immediate symptoms like those seen in anaphylaxis, and four of these died within a few minutes with typical features of anaphylactic death and with characteristic necropsy findings.

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[19] Neufeld and Dold: Berl. klin. Wchnschr., 1911, xlviii, 1069.
[20] Rosenow: Jour. Infect. Dis., 1911, ix, 190.
[21] Cole, Rufus: Jour. Exper. Med., 1912, xvi, 644.

From the experiments it is evident that the development of the toxic substance is more constant and striking in the peritoneal cavity of the guinea-pig than it is in the test-tube. In the animal body, however, conditions are complex and it is difficult to know whether the toxic substance is specific or bears any direct relation to the infectious agent. We therefore tried to obtain solutions of the pneumococcus bodies by other means. Making extracts in chloroform and in ether did not yield solutions that could be readily studied. We next studied the solution of pneumococci obtained by means of bile. In making the solutions a 2 per cent. solution of sodium cholate in normal salt solution was employed. The effect of the intravenous injection of bile extracts of pneumococci has now been tested in a very large number of guinea-pigs and rabbits. In a large proportion of cases death with acute symptoms resembling those in anaphylactic shock occurs. When smaller amounts of the extract are injected, or when the toxicity of the extract is less, the animals die in from two to twelve hours. Such animals usually show more or less pulmonary edema and hemorrhages, and small hemorrhages are present in the peritoneum and diaphragm and in the walls of the stomach and intestines.

It is probable, from the effects produced, that the substances operative here are the ones that produce the effects in "anaphylatoxin" and in the salt solution extracts. In the latter case it has been assumed by Rosenow that the toxic substances result from the digestion of the bacterial protein by the ferments contained in the bacterial cell. The proof of this, however, does not seem convincing. The fact that the solution of the pneumococci in cholate solutions may occur within one-half hour at 4 C. (39.2 F.) is evidence that in this case the active substance is not the result of ferment action. In a recent communication Jobling and Strouse[22] have presented good evidence to show that the lysis of pneumococci in salt solution is probably not merely the result of ferment action. All these experiments indicate that the bodies of pneumococci contain substances which are toxic when they are set free by the solution of the bacterial bodies. They therefore present evidence in favor of the well-known endotoxin theory of Pfeiffer.

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[22] Jobling and Strouse: Jour. Exper. Med., 1913, xviii, 597.

During the past few years this theory of the origin of toxic substances has been largely neglected, owing to the interest in the theories of Vaughn and Friedberger, according to which the intoxication in all forms of infection is caused by substances which are intermediate products in the digestion of protein. Vaughn goes so far as to state that the substances producing the symptoms are identical in all infections and that the different symptom-complexes are dependent, not on the nature of the intoxicating substance, but on other conditions. It would hardly seem, however, that the intoxicating substance causing the rapid pulse and rapid, labored respiration and violent delirium of pneumonia is identical with the intoxicating substance in typhoid in which there is a relative slowing of the heart and low, muttering delirium. Though the intoxication may be due to the products of protein digestion, it does not necessarily follow that the substance is the same in all cases, as the bacterial proteins must differ enormously in composition.

While the obtaining of toxic substances from the bodies of pneumococci is of great interest, it is quite evident that this, in itself, does not contain the proof that we are dealing with the substances responsible for the intoxication in pneumonia. In order to present such evidence, further knowledge is required of the nature of the substance and especially of its relation to pneumococcus immunity.

Certain facts have already been established in regard to this toxin. Its study has been greatly facilitated by the fact that when added to washed sheep-corpuscles hemolysis occurs. So far as studied, the toxic effects are caused by the same substance which produces hemolysis, since the two properties are influenced by the same measures and vary in equal degree. One of the most important facts that has been determined in regard to this toxin is that the toxic and hemolytic properties vary with the virulence of the organism employed. Extracts from non-virulent cultures, so far as studied, are not toxic. The substance which is responsible for the formation of methemoglobin in the body and the discoloration of blood in cultures, however, does not seem to be present in this toxin. The toxin is destroyed by heating one-half hour at 56 C. (132.8 F.). This may explain why the injection of pneumococci killed by heat produces no effect in the animal injected. It loses its toxicity when kept for twenty-four hours at 37 C. or for two or three days on ice. It may be dried, in which condition its toxic properties disappear much more slowly. It does not pass readily through a Berkefeld filter and it is precipitated by colloidal iron solutions. Many attempts have been made to neutralize its action by the use of dyes, by nucleic acid, nucleates, glycocoll, glycuronic acid, etc., substances which are considered to attach themselves to toxic substances in the body and thus to render them non-toxic. None of these experiments have been successful. The only substance so far found which is able to neutralize the effect of the toxin is cholesterin. When cholesterin is mixed with the toxic substance and kept at 37 C. for fifteen minutes, the toxic effect, as shown by injection into animals, and also the hemolytic effect, is lost. When the toxin is mixed with cholesterin and immediately injected into the animal, however, or when the toxin is first injected and is immediately followed by the cholesterin, the toxic effects cannot be prevented. Nor can the toxic effects be prevented by injecting the cholesterin before the toxin. The most important results in this study have been obtained in the attempts to produce antiserums to these toxins, and of these I shall speak later.

Whatever may be the mechanism by which intoxication is brought about, have we any evidence as to the determining factors in the final outcome, that is, as to why the patient recovers or dies? The results of our blood-cultures would seem to indicate that the occurrence of septicemia plays an important part in the death of the patient. A study of the virulence of the cultures from the blood also seems to show that the intoxication is greater and the prognosis worse when the organisms have a high virulence than when they have a low virulence. Moreover, our clinical experiments seem to indicate that the progressive extension of the local lesion is of bad prognostic import; that the failure of the body to erect a limiting barrier to the local extension of the disease is an important factor in the fatal outcome. At any rate, it is certainly true that in most fatal cases, on examining the lung, one sees, not a sharply localized lesion, but an extending lesion frequently involving several lobes. This progressive extension seems to bear some relation to the virulence of the organism. With organisms of low virulence, the body is able to resist the infection, as regards both the spread of the local lesion and the general infection.

We have made quite extended studies to learn something of the nature of the general resistance of the body to the pneumococcus infection and its effects. It would seem that in pneumonia with its sudden crisis—one of the most startling and dramatic events with which the physician has to deal—an ideal opportunity would be offered to learn the nature of the process of recovery. It must be borne in mind, however, that only in certain cases does such a critical change in the patient occur. Of about 10,000 cases collected by Musser and Norris[23] the temperature fell by crisis in only about half. In the other cases it is difficult to determine with accuracy just when the change in the patient's condition occurs. It is therefore a mistake to think that in pneumonia we have a sudden change from susceptibility to resistance. More likely the process is a gradual one, and the marked change in the patient's condition occurs when the resisting factor, which increases gradually, reaches a degree sufficient to be effective. This factor of resistance may not be a single one, but the result may be due to a summation of several factors.

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[23] Musser and Norris: In Osler's Modern Medicine, Philadelphia, 1907, ii, 537.

It has been suggested that the crisis represents a kind of anaphylactic reaction. It is known that following serum anaphylaxis there occurs a period during which the animal is in a refractory state. If the intoxication in pneumonia is due to peptone-like substances derived from the bacterial protein, it is possible that the crisis is a form of cumulative shock, following which the patient is refractory. Little is known, however, concerning such prolonged anaphylactic intoxication, and the nature of antianaphylaxis is still so obscure that it does not seem profitable to dwell longer on this theory, attractive as it is.

The fact that the crisis usually occurs in about seven days is strongly suggestive that the reaction is a true immunity reaction, since it is about in this time that antibodies appear in the blood in their maximum concentration, as we know from artificial immunization.

The view that recovery in pneumonia is due to the production of immune substances presupposes that at the end of an attack of pneumonia the patient is immune. We know from experience, however, that this is not so, or if immunity is present, it is of very short duration. I have seen a patient return to the hospital with a typical attack of pneumonia two days after discharge from a previous attack. Moreover, it is well known that a person may have repeated attacks; in fact, one attack seems to render a person more susceptible. It is quite possible, however, that the relative natural immunity of man requires only a very slight assistance in the shape of acquired humoral immunity in order to render the body able to overcome the infection, and following this the immune bodies may very quickly disappear from the blood. The attempts to demonstrate the appearance of known immune substances in the blood during and following an attack of pneumonia have not previously been very successful. An increase of the ordinary bactericidal substances which act in conjunction with complement has not been proved. Most observers have found that the pneumococci grow quite well in the blood-serum of patients recovering from pneumonia, even in the serum of immunized animals.

It has been asserted that by combining the leukocytes and serum of pneumonia patients, or by using the defibrinated blood, definite differences may be demonstrated between the blood of normal persons and that of patients during or following the crisis. None of these experiments seem to me free from objections. There does not seem to be sufficient evidence for the conclusion that the recovery is due merely to an increase of opsonins, though Clough,[24] who has studied the phagocytic activity of the serum obtained after crisis or lysis in a series of eleven cases, found in six of these definite power of the serum to bring about phagocytosis of virulent pneumococci. In two of these cases the serum was tested before crisis and showed no such action. In his experiments, with one exception, the phagocytic activity was limited to the homologous strain. It has been stated by Rosenow that a difference exists as regards phagocytic activity between the leukocytes of patients with pneumonia and those of normal persons, though the results of others (Tunnicliff and Eggers) do not confirm these conclusions.

TABLE 1.—Protective Power of Serum of an Untreated Patient—

Quantity of Culture in c.c.	Quantity of Serum in c.c.	Virulence; no Serum	Control; Normal Serum	Three Days Before Crisis	Two Days Before Crisis
0.1	0.2	—	—	—	24†
0.01	0.2	—	—	—	42†
0.001	0.2	27†	42†	42†	29†
0.0001	0.2	42†	42†	66†	42†
0.00001	0.2	50†	42†	32†	42†
0.000001	0.2	45†	43†	42†	47†

* Animals protected as shown by survival. † No. of hours before death of animal

Wolff[25] has attempted to show the increase of phagocytic power in the blood of pneumonia patients by making a composite curve combining the number of leukocytes with the opsonic index. We feel, however, that the errors in the usual opsonic technic are too great to justify his conclusions.

Of more importance are the experiments showing an increased protective power for mice of the blood of patients after recovery from pneumonia, as tested against known lethal doses of pneumococci. Neufeld has shown that while normal human serum had no protective action, that obtained from certain patients following the crisis had a definite effect. Strouse,[26] and Seligmann and Klopstock,[27] however,

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24. Clough: Bull. Johns Hopkins Hosp., 1913, xxiv, 295.
25. Wolff: Jour. Infect. Dis., 1906, iii, 731.
26. Strouse: Jour. Exper. Med., 1911, xiv, 109.
27. Seligman and Klopstock: Ztschr. f. Immunitätsforsch., Orig., 1909-10, iv, 103.

failed to demonstrate such changes. Studies on this question were therefore undertaken by Dochez[28] on a series of cases. The method used was the following: Specimens of the patient's serum were obtained on various days both before and following the crisis. When possible, the organism against which the serum was to be tested was cultivated from the patient, either from the blood or sputum. In case the pneumococcus, when isolated, was of low pathogenicity, the virulence was raised by successive animal passages until a dose of 0.000001 c.c. of a broth-culture was sufficient to kill. Twenty-four-hour broth-cultures fresh from animals were used for infection, and the serum and varying quantities of the culture were mixed in the barrel of a syringe and immediately injected intraperitoneally. The appearance of protective substances in the blood could then be detected, as shown by the protocol from one such experiment (Table 1).

—with Lobar Pneumonia at Varying Stages During the Disease

Serum Obtained						
One Day Before Crisis	Three Hours After Crisis	Two Days After Crisis	Four Days After Crisis	Five Days After Crisis	Seven Days After Crisis	Eight Days After Crisis
24†	32†	29†	42†	42†	32†	24†
20†	75†	42†	42†	27†	—	32†
42†	66†	*	*	*	42†	42†
66†	*	*	49†	42†	44†	42†
42†	*	*	*	42†	66†	45†
66†	*	*	*	*	45†	96†

injected.

The serums from fourteen cases of pneumonia were so studied. In ten of these the serums were tested against homologous organisms. Of these ten cases all but one showed at some time the appearance of protective substances in the blood. Of the serums from four cases tested against stock cultures, only one showed any protective power. The amount of protection was never very high, though in some instances 0.2 c.c. of serum were able to protect against one thousand times the minimal lethal dose. The time of appearance of the protective substances varied somewhat, though in seven instances protective substances either appeared for the first time or showed a marked increase in amount at the time of crisis or, in case of lysis, during the period when the symptoms were abating. In two cases the serum taken during the period of defervescence exhibited little or no power of protection, even against homologous strains, and it was not until some time later, in one case sixteen days, that the presence of protective substances in the blood was demonstrated.

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[28] Dochez: Jour. Exper. Med., 1912, xvi, 665.

Clough[24] later carried out a similar set of experiments, and in nine out of twelve cases the serums after crisis or lysis showed definite protective power against homologous strains. The technic used differed somewhat from that employed by Dochez and the results were not so striking, but show well that in most cases the serum acquires definite protective power.

These experiments are of great importance as showing, first, that in many cases, at least, protective substances appear in the blood of patients recovering from lobar pneumonia, and second, that these protective substances in many cases are active only against the race of organism concerned in the infection. These experiments, however, do not yet establish that crisis or recovery in pneumonia is due alone to the development of these protective substances in the blood. As already stated, in certain cases they cannot be demonstrated. It is altogether probable, however, that they play some part in the final outcome. As to the nature of the substances which are most active, it is impossible at the present time to state.

Probably recovery from pneumonia occurs when the growth of the organisms is inhibited and their toxic effects neutralized. It is impossible to state which comes first. It is conceivable that if the toxic effects of the bacteria are neutralized, the body is readily able to cause their destruction, since it is possible that pathogenicity depends entirely on toxicity. There is some evidence, as I shall show, that immune serums are antitoxic. With the present technic it has not been possible to demonstrate increase of antitoxic power of the patient's serum during the crisis.

In the immune-body theory of the crisis, the local lesion is left entirely out of consideration. It is quite evident that in pneumonia we are dealing, not merely with a septicemia, but with a condition in the lung which has a very important bearing on the termination. The involved portion of the lung forms a solid mass in which are growing numbers of micro-organisms. In each alveolus are fibrin, leukocytes, red blood-corpuscles and bacteria, and in the spaces free serum. Now it is known that as the process advances, the number of leukocytes becomes greater and greater. Resolution finally occurs almost certainly as a result of this increase and associated breaking down of the leukocytes, and with this the setting free of ferments which bring the fibrin into solution. The fact that this does not occur earlier is due to the overbalancing of the leukocytic ferments by the antiferments of the serum, and the lytic ferments become active only when the relation between leukocytes and serum is in favor of the former. It is conceivable that recovery only ensues when such a balance occurs and when with the solution of the fibrin, tension is relieved and there is an outlet for the exudate. Instead of the surgeon inserting a knife, nature does the work by injecting a ferment.

It is quite probable, moreover, that during resolution other factors than the purely mechanical are at work. With the solution of the exudate, numerous substances are formed which have a direct destructive action on the bacteria. Such substances as the soaps of fatty acids, which are known to have such a destructive action, have been demonstrated in the resolving lung by Lamar.[29] Moreover, it is well known that during the growth of pneumococci outside the body, substances are formed in the culture medium which themselves are destructive. It is quite probable that such substances are being formed in the lung and they may aid in bringing about destruction of the pneumococci. Against the view that crisis depends mainly on resolution of the exudate, however, may be brought the very evident and conclusive objection that they do not necessarily occur synchronously. Resolution may be long delayed, or resolution may be occurring in one part of the lung while the process is advancing in another.

That leukocytes play some part in recovery is rendered probable by the experiments of Klein and Winternitz.[30] They have shown that when rabbits are treated with benzene, a leukotoxic substance, the animals rapidly succumb to pneumococcus infection, whereas when they are treated with toluene, which is a similar substance but which has no effect on the leukocytes, no decreased resistance is seen. Whether the chief function of the leukocytes consists in limiting the local infection, in which they undoubtedly play a rôle, or in aiding in the development of a general immunity is not indicated by these experiments. Clinicians, however, have long been of the opinion that a low leukocyte content of the blood is unfavorable.

It is not unlikely that in recovery all of the factors mentioned play a part. The destruction of the bacteria in the lung lesion may depend on local factors quite different from those responsible for the destruction of the bacteria in the circulating blood. From present knowledge it would appear that the growth of bacteria in the blood is the most serious part of the pneumonic process, and it seems that this, at least, is influenced by the appearance of circulating anti-bacterial substances.

METHODS OF CURE

It has been known since 1891 that susceptible animals may be rendered resistant to the action of pneumococci by the injection of increasing and properly spaced doses of pneumococci, beginning with the dead organisms. Moreover, it was early shown that if a very small amount of the serum of the immunized animal is injected into a second animal, this animal for a short time is also immune. These experiments are so striking and fundamental that it is no wonder that various attempts have been made to prepare and use such serums therapeutically. The clinical results, however, have not been convincing. Certain observations made principally by Neufeld and his collaborators and other observations made in our own laboratory, suggest reasons why such results have not been satisfactory and methods for overcoming the difficulties.

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[29] Lamar: Jour. Exper. Med., 1911, xiii, 1.
[30] Klein and Winternitz: Jour. Exper. Med., 1913, xviii, 50.

Opinions have differed as to whether or not an immune serum produced by the injection of a given race of pneumococci into an animal is effective against all races of pneumococci. The first accurate studies on this problem were made by Neufeld and Händel.[31] They tested a so-called univalent serum against various races of pneumococci. While this univalent serum was protective in mouse experiments against fifteen strains studied by them, against other strains the serum had practically no effect. They decided that these atypical strains were not *Serum-fest* in the ordinary sense of the term, since the serum obtained during convalescence from one of the patients, from whom one of these organisms had been isolated, protected mice against the homologous strain and also against one of the other atypical strains, but did not protect against the typical strain. They then produced an immune serum against one of the atypical strains to see whether all atypical strains could be affected by this immune serum, but found this not to be true. In their further studies they found that the second immune serum, which they called *Serum Franz* protected against only three of the atypical strains isolated by them, but failed to protect against three other strains. These latter three strains they further showed to be individual in their reactions. Neufeld and Händel did not have access to a large number of patients with pneumonia from whom to obtain cultures, and could not determine the frequency of occurrence of atypical types, nor could they make extended studies on grouping of the organisms on a biological basis, though from their studies the possibility of making such a grouping was most evident.

With the opening of the Hospital of the Rockefeller Institute in October, 1910, patients suffering from lobar pneumonia were admitted for treatment and study, and an extended study was commenced of the pneumococci obtained in these cases. An immune serum was prepared by injecting a horse with a culture of pneumococcus obtained from Professor Neufeld, the same race he had employed in the production of his immune serum. The protective power of this serum for mice was tested against a number of races of pneumococci cultivated from a series of cases of pneumonia. A report by Dochez[33] gave a preliminary report of this study, indicating that this serum protected against only about half the races studied. It was therefore evident that if such a serum were employed therapeutically, an effect could be expected at the most in only about half of the cases treated.

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[31] Neufeld and Händel: Ztschr. f. Immunitatsforsch., 1909, iii, 159; Arb. a. d. k. Gsndhtsamte, 1910, xxxiv, 169; ibid., 1910, xxxiv, 293; Berl. klin. Wchnschr., 1912, xlix, 680.

Experiments[34] were then undertaken to determine whether it would be possible to make a biologic classification of pneumococci obtained from cases of pneumonia, based on their reaction to different serums in protection experiments. Rabbits were therefore immunized to each of the races which were not acted on by the horse-serum, which we have called Serum 1, and the protection afforded by these different rabbit serums against all the other races tested. A considerable number were found to show cross-protection, that is, a serum prepared by injection of one of the number acted on all the races of this group. A horse was then immunized to one of this group and the serum is called Serum 2. In this way it has been possible to divide the pneumococci obtained from cases of pneumonia into four groups. In Group 1 are included all the races against which Serum 1 is effective. In Group 2 are included all those against which Serum 2 is effective. Whether the races included in this group correspond with the organisms described by Neufeld as acted on by his immune serum *Franz* is not known at present. In Group 3 are placed all the organisms of the so-called *Pneumococcus mucosus* type. These organisms have very large capsules and produce a sticky exudate in animals. In Group 4 are included all the races against which Serums 1 and 2 are not effective and which, from their other properties, do not belong in Group 3. Animals may readily be immunized to any member of this Group 4, and the serum of the immunized animal is protective against the race used for immunization. In no instance, however, has this serum been found effective against any other race of this group or against the organisms of the other groups. So far as cultural and morphologic characters are concerned, no constant group differences have been discovered between the members of Groups 1, 2 and 4. By means of the agglutination reaction, however, it has been found possible to group them in exactly the same manner as by protection experiments.

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[33] Dochez: Jour. Exper. Med., 1912, xvi, 680.

[34] Dochez, A. R., and Gillespie, L. J.: A Biologic Classification of Pneumococci by Means of Immunity Reactions, Jour. Am. Med. Assn., 1913, lxi, 727.

As previously stated, the members of Group 3 differ from the others somewhat in their morphologic and pathogenic characters. They differ further in the fact that while animals may be very highly immunized to them, the serum of such animals possesses no protective power; they induce active but no passive immunity. Studies have been undertaken by Hanes to learn on what factor this failure to produce passive immunity depends. It was found that the serum of the immunized animals not only does not protect, but also has no agglutinating power. It has been known that certain encapsulated bacilli also fail to be agglutinated by immune serum. Porges,[35], [36] however, has shown that such bacilli are agglutinated by the serum of immunized animals, provided the bacilli are previously treated so as to destroy their capsules. This method was therefore employed by Hanes[37] in studying these cocci. Six typical races of *P. mucosus* obtained from cases of pneumonia, were studied. The bacteria were treated with dilute hydrochloric acid and heated for fifty minutes at 80 C. (176 F.). The fluid was then neutralized and the bacteria so treated tested for agglutination. Controls were made with members of Groups 1 and 2 treated in the same way. The results were definite and striking. Agglutination of all the six races of *P. mucosus* occurred promptly with all six immune serums obtained by inoculating each of a series of rabbits with one of these races. No agglutination of Pneumococcus 1 or 2 occurred with any of these serums, and *P. mucosus* was not agglutinated by either Serum 1 or 2. (See Table 2).

TABLE 2.—Agglutination of Pneumococcus Mucosus

Organism No.	Immune Serums Nos.								Normal Rabbit Serum
	19	26	42	54	68	96	I	II	
19	+	+	+	+	+	+	—	—	—
26	+	+	+	+	+	+	—	—	—
42	+	+	+	+	+	+	—	—	—
54	+	+	+	+	+	+	—	—	—
68	+	+	+	+	+	+	—	—	—
96	+	+	+	+	+	+	—	—	—
I	—	—	—	—	—	—	+	—	—
II	—	—	—	—	—	—	—	+	—

These experiments show that, so far as tested, all the organisms of the *P. mucosus* type belong in one biological group differing from

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35. Porges: Wien. klin. Wchnschr., 1905, xviii, 691.

36. Porges and von Eisler: Centralbl. f. Bakteriol., First Abt., Orig., 1906, xlii, 660.

37. Hanes: Jour. Exper. Med., 1914, xix, 38.

those of the other groups. In order to show the relation of these organisms to streptococci, the method of complement-fixation was employed. With this method there occurred a considerable amount of cross-fixation among the various races of pneumococci, including *P. mucosus,* but no cross-fixation was observed in testing the complement-fixing powers of *Streptococcus mucosus* or *S. pyogenes* serums. It therefore seems evident that *P. mucosus* is really a variety of pneumococcus, and that biologically it forms a distinct variety of this organism.

Further studies of the various members of the *P. mucosus* group, to see if any were affected by the immune serum *in vivo,* were all negative. None of the serums were able to protect mice, even against the homologous organism. These experiments and also observations of Gruber and Löhlein seem to indicate that the failure of such serums to protect is in some way related to the formation of the thick, mucoid capsules by these organisms. As soon as the bacteria commence to grow in the body, capsules are formed which prevent the action of the immune serum. By the methods employed by Dochez and Hanes, it has been possible to study the races of pneumococci obtained from a series of cases of pneumonia. The classification by protection and agglutination experiments of sixty-two organisms so obtained gave results as shown in Table 3. In every instance in which an organism could be placed by protection in one of the groups described, the agglutination reaction has corresponded.

TABLE 3.—Classification by Protection and Agglutination

	Number	Per Cent.
Group 1	35	47
Group 2	13	18
Group 3 (P. mucosus)	10	13
Total typical	58	78
Group 4 (heterogeneous)	16	22
Total heterogeneous	16	22
Total number	74	

The races placed in Group 4 have been called heterogeneous, since each race, so far as studied, appears independent, and no grouping of the members on a biologic basis, by means of protection or agglutination, is at present possible. Table 4 shows the results of the study of agglutination with these races.

Gillespie[38] has also made a study of the various races, using the method of agglutination of bacteria by acid, as introduced by Michaelis. The results also show certain group differences in the agglutination of the various races.

Recent observations by Rosenow indicate that by certain methods it is possible completely to change the characters of the organisms of the entire streptococcus-pneumococcus group, so that one may be transformed into the other, even *S. hemolyticus* into a typical pneumococcus and vice versa. It has long been thought that the various closely related bacteria must originally have had a common source and have become differentiated by processes of adaptation. It is remarkable, however, that the changes can occur in such a short period of time as shown by Rosenow, even though they are subjected to extreme changes in environment, as has apparently been done. Some experiments performed in my laboratory several years ago by Strouse indicated that sudden mutations might appear in this group. Important as these experiments are, they do not have an immediate bearing on the pneumonia problem, except as regards the origin of the infection.

TABLE 4.—Strains of Heterogeneous Group Tested and Results of Agglutination

Serums Pneumococcus	Pneumococcus Strain Numbers													
	1	2	34	36	37	38	52	55	60	62	71	76	82	102
1	+	0	0	0	±	0	0	0	0	0	0	0	0	0
2	0	+	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	+	0	0	0	0	0	0	0	0	—	0
37	0	0	0	0	+	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	+	0	0	0	—	0
71	0	0	0	0	0	0	0	0	0	0	+	0	—	0
76	0	0	0	0	0	0	0	0	0	0	0	+	0	0
82	0	0	0	0	0	0	±	0	0	0	0	0	+	0
Polyvalent, 34, 38, 62.	0	0	0	0	0	+	0	+	0	+	0	0	—	0

As regards the course of the disease and the possibility of specific methods of cure, the possibility of transformation of type of the organism concerned is not significant. In all the studies of organisms obtained at different times from the same case, in no instance have there been any indications of a change in type; the type first isolated has always subsequently been found. Moreover, many of these strains have now been cultivated for a long time outside the body, both in artificial mediums and in repeated passages through animals, some of them for several years, and they have in all cases retained their original characteristics. The results of the present year are not included in Table 3. This year the largest number of cases has been due to organisms of Type 2. It is possible that the prevalence of cases due to the various types varies from year to year and in different places, which may explain the variation in mortality observed in different times and places. The mortality of our limited number of cases due to organisms of different types is shown in Table 5. The most striking fact is the low mortality due to organisms of Type 4. Further observations may possibly change our ideas with regard to the relative severity of cases due to organisms of the different types.

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[38] Gillespie: Jour. Exper. Med., 1914, xix, 28.

In addition to the fact that there are immunological differences in the pneumococci concerned in pneumonia, there is probably another reason why the use of immune serum has not proved efficacious in the past. The method of employment of such immune serum has been to use small doses, from 10 to 20 c.c., usually given subcutaneously. Neufeld and his assistants, by titrating immune serum against varying doses of pneumococci and making injections into mice, have concluded that in order to obtain protective power a certain proportion of serum in relation to body-weight is required. This concentration they have called the *Schwellenwert* or threshold concentration, and from experiments on mice they estimate that for man the dose of serum employed by them must be at least 77 c.c. Undoubtedly, however, this *Schwellenwert* must vary enormously under different conditions, depending on the virulence of the organism, the time the serum is given, etc. In any case their experiments indicate that the serum must be given in very much larger amounts than has hitherto been done, in order to obtain curative results. They concluded that such an antibacterial serum does not obey the law of multiple proportions. This is undoubtedly true for the conditions employed by them, namely, injections made separately in different parts of the body, and is also true for the conditions present

TABLE 5.—MORTALITY IN PATIENTS INFECTED WITH VARYING TYPES OF ORGANISMS

Infection Type	No. Patients	Patients Died	Per Cent.
1	34	8	24
2	13	8	61
3	10	6	60
4	15	1	7
Total..........	72	23	32

in the therapeutic injections in man. Dochez has shown, however, that when the serum and cultures are mixed before injection, such a serum does obey the law of multiple proportions up to a certain point, but there is a maximum degree of infection against which no amount of serum, however large, is able to protect. It would therefore seem that one of the factors of the protective mechanism must be supplied by the body, and that, when the infection is very great, a sufficient number of immune bodies may be supplied by the administration of serum, but the body cannot react to a sufficient extent adequately to supply this second factor. This suggests that in order to obtain results from serum, it should be administered early, before the infection has reached too extreme a grade, beyond which no amount of serum can be effective, and also offers a possible explanation of the fact that in certain cases, such as the one which I shall mention, case 4, the serum seems to have absolutely no effect. The nature of this additional factor is not known. If, as previously stated, the serum owes much of its effect to its bacteriotropic power, the number and activity of the leukocytes may be the additional factor. That this factor may be stimulated is shown by the results of artificial immunization, whereby very much larger doses of bacteria are resisted than can be protected against by immune serum.

These experiments have indicated that for the successful employment of immune serum in pneumonia, it must be employed fairly early and in large doses, and a serum must be used which is effective against the variety of organism causing the infection. We have been able to produce a serum of very great efficiency against organisms of Type 1, and one effective against organisms of Type 2. So far it has been impossible to produce a serum effective against *P. mucosus,* and, for the reasons stated, it would only be practical to treat cases of pneumonia due to organisms of Type 4 with homologous serums. This is of less importance, however, since the cases due to these organisms are apparently of mild grade.

To employ the serum effectively in cases due to organisms of Types 1 and 2, it has been necessary to devise a method for quickly determining in each case the type of organism concerned. This has been done and the following method is employed. When a patient with pneumonia is admitted to the hospital, a culture is immediately made from the blood and also one from a portion of sputum coughed up from the lung, or, when this is not obtainable, a culture is made directly from the lung by the insertion of a needle. This procedure seems to be without danger. When there are large numbers of organisms in the sputum, a culture may be obtained most rapidly by injecting the washed sputum into the abdominal cavity of a mouse. After four or five hours the peritoneal cavity may be washed out with salt solution and the cells thrown down in the centrifuge; a suspension of the organisms is thus obtained. In whatever way the culture is obtained, the agglutination test is at once applied. If the organism fails to agglutinate with either Serum 1 or Serum 2, it is, of course, useless to undertake serum treatment. If, however, one of the serums agglutinates the organism, treatment may be commenced at once with the appropriate one.

So far it has been possible to treat only a comparatively small number of cases. Twenty-three cases have been treated with the serum. Of these, fifteen were due to organisms of Type 1 and eight to organisms of Type 2. The method of administration of the serum was the following: When admitted, the patient was given 0.5 c.c. of serum subcutaneously to discover if hypersensitiveness existed. As soon as the type of organism was determined, from 50 to 100 c.c. of the serum, diluted one-half with salt solution, were injected intravenously. The condition of the patient served as a guide in the later

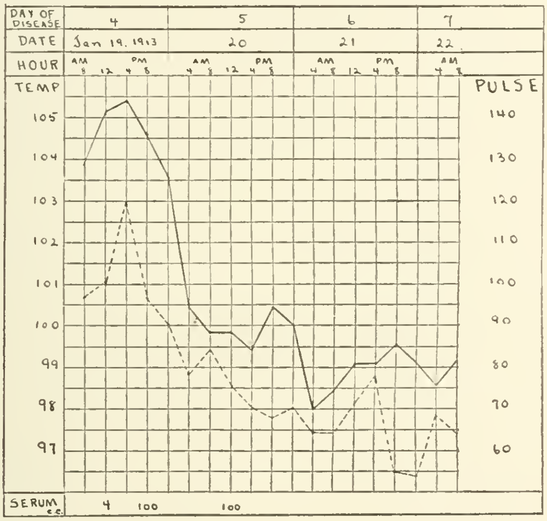

Fig. 1.—Case 1, E. S., No. 988, aged 20 years, admitted January 18, 1913, on the third day of the disease. Physical signs showed involvement of the lower left lobe and also signs of beginning involvement in the lower right lobe. Blood culture was negative. Pneumococci obtained from sputum were of Type 1. The patient was quite ill on admission, respirations were labored and there was cyanosis. His condition was markedly improved on the day following the first administration of the serum, as shown by the temperature and pulse curves. Recovery was uneventful except that slight signs in the lungs persisted for several weeks.

treatment. Usually the serum was not administered oftener than every twelve hours. The patients treated received totals of from 190 to 460 c.c. of serum, except one, who received a total of 700 c.c. of serum. The patients treated were all seriously ill. They were treated in series. Every case infected with a pneumococcus of Type 1 or Type 2 was treated. Of the fifteen cases due to Pneumococcus 1, all of the patients recovered but one, and of the eight cases due to Type 2, two patients died. One of these patients objected to the treatment and would not allow its continuation, so it was not thoroughly carried out. When we consider that the mortality among the untreated patients infected with Types 1 and 2 is very high (Table 5), the result is certainly not discouraging. It must also be remembered that so far most of our cases have been admitted late in the disease. Treatment was commenced on the third day in six cases, on the fourth day in five cases, the fifth day in six cases and the sixth day in six cases. If treatment can be commenced early, it is probable that the results will be even better than they now are. It is to be hoped that during this winter a large number of cases may be treated early in the disease. Effective treatment in the cases due to Types 1 and 2 should cut down the total mortality due to pneumonia very materially, as it has already done in our hands. I prefer at present, however, not to lay the main stress on the mortality statistics, since these are not large enough to be conclusive, but to refer to other criteria which we possess as to the efficacy of the serum.

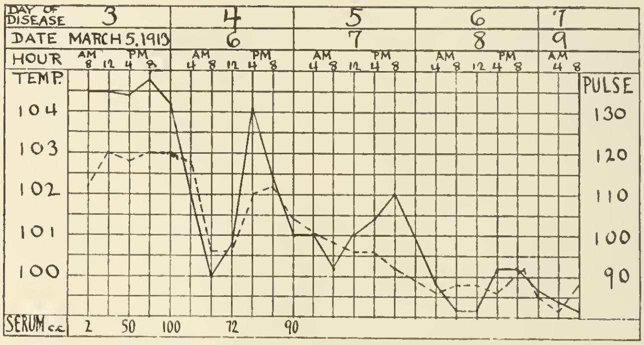

Fig. 2.—Case 2, B. G., No. 1175, aged 36 years, admitted March 4, 1913, on the second day of the disease. There was slight involvement at the base of the right lobe. Blood culture was positive. Agglutination test showed Type 1 organism. Treated with serum on day following admission. The signs of involvement in the right lower lobe became more distinct, but there was no apparent extension of the involvement beyond this lobe during the course of the disease. Following injection of the serum the patient's general condition improved. The patient complained of some urticaria and joint pains beginning on the twelfth day.

Let us first consider the effect on the clinical course of the disease. Following practically all the injections, a reaction has occurred. The temperature usually rises and then falls, but does not necessarily remain low. In two instances the rise of temperature has been marked. In the other cases the rise of temperature following the injection was only a degree or so. In all the cases except the fatal ones, the serum has apparently had an ultimate favorable effect in lowering the temperature and shortening the course of the disease, though, of course, this is a very difficult matter of which to be absolutely sure. In no case was one injection of the serum sufficient to bring on a crisis.

Figures 1, 2, 3 and 4 show the effect of treatment on the temperature curves in certain of the cases. It is manifest that wrong impressions may be produced by the exhibition of temperature curves unless all the curves of a series are given. To avoid this difficulty, so far as possible, however, a curve from each group of cases is shown. Figure 1 represents the curve of a case in which the serum apparently had a marked effect, the temperature falling promptly and in a striking manner. Figure 2 indicates a temperature curve in a case in which the temperature fell following the administration of serum, but several doses were necessary before the temperature remained low. Figure 3 shows the temperature curve in a case in which there were apparent

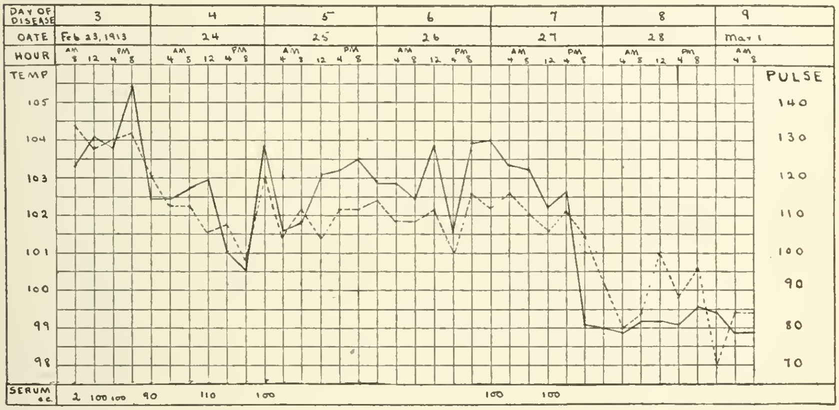

Fig. 3.—Case 3, M. L., No. 1091, aged 30 years, admitted February 22, 1913, on the second day of the disease. Blood culture showed a growth of pneumococcus, Type 1. Physical signs indicated involvement of the right lower lobe with slight involvement of the left lower lobe. The patient's condition improved following the treatment and the signs in the lungs became less well marked. On the sixth day, however, the patient's condition was again worse, the temperature was higher and the pulse more rapid, so that treatment was commenced again, following which the patient's condition markedly improved and he made a rapid recovery except for symptoms due probably to serum sickness on the twentieth to the twenty-fifth days of the disease.

effects of the administration of serum, but after the administration of serum was discontinued, the temperature curve rose and only fell after further large doses of serum were administered. In Figure 4 is given a temperature curve in which the serum had apparently no effect and the patient died on the seventh day.

All the patients seemed to feel better following the injection of the serum, and in a number of cases the apparent lessening in the degree of intoxication was very manifest. When the treatment was commenced early, no extension of the involvement of the lung occurred. On the other hand, there was no special tendency in the treated cases for the lung lesion to resolve rapidly. If anything, there seemed to be a tendency for resolution to be delayed in these cases. This has been noted by others in certain cases treated by serum.

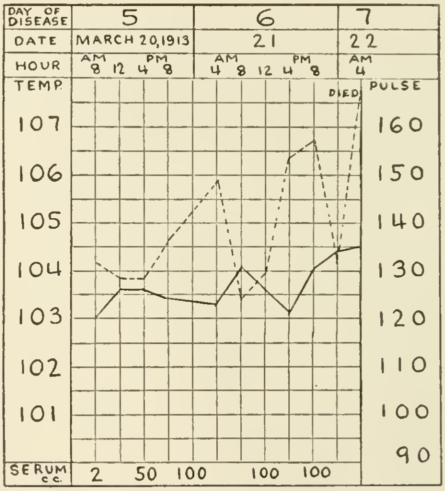

Fig. 4.—Case 4, F. W., No. 1090, aged 36 years, admittted March 19, 1913, on the fourth day of the disease. The patient was extremely ill on admission. There was involvement of the right upper and lower lobes. Blood culture and culture from the lung showed the presence of pneumococcus, Type 1. There was some irregularity of the heart. Following treatment on the fifth day, the patient seemed better. The blood culture was negative. The pulse continued rapid and showed an extrasystolic irregularity, however, and the patient showed a very marked cyanosis. In spite of the active serum treatment, the patient died on the seventh day.

More important than the foregoing criteria, however, as indicating an effect of the serum, are the following observations, since they have depended solely on objective procedures: First to be mentioned is the effect of the serum on the organisms in the blood. In ten cases pneumococci were isolated from the blood before the treatment was commenced. In all cases blood-cultures were made before each treatment; and in all of these ten cases after one treatment and before the second (or within from eight to twelve hours) the blood had become sterile. The conclusion seems justified, therefore, that one large dose of active serum given intravenously is sufficient to sterilize the blood. It also seems certain that if the organisms are not present in the blood, the administration of the serum will prevent their invasion. Second, in a previous study of the protective substances in the blood-serum of patients with pneumonia, it has been shown that, as a rule, the appearance of protective substances in the blood, when demonstrable, coincides rather sharply with the period of critical fall in temperature and the disappearance of symptoms. Before the crisis they are not present in the blood in any measurable degree.

TABLE 6.—PROTECTIVE POWER OF SERUM B. G.; TREATMENT COMMENCED ON THE THIRD DAY

Quantity of Culture in c.c.	Virulence; No Serum	Quantity of Serum in c.c.	Serum Obtained			
			Third Day, Before Treatment	Third Day, 6½ Hours After Treatment	Fourth Day, After Treatment	Ninth Day, Five Days After Last Treatment
0.001	—	0.2	16†	*	*	† Days
0.0001	—	0.2	20†	† 5 Days	*	*
0.00001	24†	0.2	24†	*	*	*
0.000001	28†	0.2	24†	*	*	*

* Animal protected as shown by survival. † Time in hours before death of animal injected.

A similar study has been made by Dochez of the protective substances in the serum in a number of the cases of pneumonia treated with serum. In all the cases studied, it has been possible to demonstrate the appearance of such substances in considerable amounts in the serum very shortly after the administration of one dose of the immune serum, even when this serum has been administered early in the disease, at a period when such protective substances are otherwise never present. (Table 6). These substances persist, and in case they play a part in the mechanism of recovery, as was concluded from the previous study, it is evident that their appearance indicates a favorable action of the immune serum.

The results obtained, therefore, from the clinical and laboratory study of this series of cases of pneumonia treated by the injection of large amounts of appropriate serum, seem to indicate that a method has been devised for the successful specific treatment of at least a portion of the cases of acute lobar pneumonia. Studies on the treatment of pneumonia by the intravenous injection of the Neufeld-Händel immune serum have been made by Beltz,[39] Weitz[40] and Geronne.[41] In none of these series, however, were studies made of the type of organisms concerned in the infection of the cases treated, and in all of the cases the amount of serum administered was too small, judging from our own experience, to be of value.

The mode of action of the immune serums is still somewhat obscure. It is quite evident that there is an antibacterial action, inasmuch as the bacterial invasion of the blood is prevented. The action on the local lesion, however, is less evident. It is probable that here the organisms can less readily be reached by the serum, though apparently in most cases the growth of the bacteria in the margins of the lesion has been inhibited, as shown by prevention of spread of the process. In addition to the antibacterial action, the clinical cases show a definite change as regards intoxication. It is possible, of course, that this is entirely associated with the destruction of the organisms. Certain experimental work, however, has indicated that the serum may possess some antitoxic effect.

When the immune horse-serum is added to the toxin obtained by dissolving pneumococci in bile, it is found that such a serum has a well-marked effect in inhibiting the hemolysis of sheep corpuscles by this toxin. When it is added to the toxin in doses of 1 c.c. of serum to 4 c.c. of toxin and placed at 37 C. (98.6 F.) for one-half hour, the effect of the toxin when injected into guinea-pigs is diminished or entirely prevented. These effects of the immune horse-serum are much less specific, however, than are the protective or antibacterial effects, since the Serum 1 acts on both Toxins 1 and 2, though most markedly on Toxin 1. Serum 2 also shows a similar diminution in specificity in antitoxic action as compared with antibacterial action. These experiments offer some evidence that part of the effect of the immune horse-serum is antitoxic, admitting, of course, that the toxic substances obtained from the bacterial bodies are responsible for the intoxication.

An effort has been made to obtain a pure antitoxic serum by the injection of the toxin alone into animals. Rabbits have been immunized by the repeated injection of this toxin and a sheep also has been highly immunized. The sheep-serum and also the immune rabbit-serums show antitoxic power, as indicated by anti-hemolytic action and also by neutralizing effect on the toxin, as tested by injection into guinea-pigs. The effects, however, are less marked than those of the antibacterial horse-serum. These antitoxic serums are also protective against the living organisms, as shown by tests on mice. The protection, however, while fairly high, is less well marked than that of the horse-serums. The protection is not so specific as that of the horse-serum, since the serum produced by the injection of toxin prepared from an organism of Type 1 is not only protective against this organism, but also, though to a less extent, is protective against organisms of Type 2.

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[39] Beltz: Deutsch. med. Wchnschr., 1912, xxxviii, 14.

[40] Weitz: Med. Klinik, 1912, viii, 1072.

[41] Geronne: Berl. klin. Wchnschr., 1912, xlix, 1699.

The interpretation of these experiments is attended with much difficulty. It is possible that these antitoxic serums may show protective power only because living organisms were introduced, since in the preparation of the toxin one cannot be positive that all organisms have been destroyed. These antitoxic serums, however, possess no power to cause agglutination, and this fact, together with their lessened specificity, suggests that we are dealing with serums which owe their power to other properties than those of the antibacterial serums. The experiments are of importance, moreover, since they indicate that immunity may be obtained against the substances contained in the bile extracts, and since the essential criterion of a toxin in the Ehrlich sense is that immunity may be obtained to it. Much more work will have to be done before such antitoxic serums should be employed therapeutically.

It is probable that in the future it will be possible to obtain the same therapeutic effects by the injection of much smaller amounts of serum than are now employed. Work now being carried on by Avery shows that the immune substances are all contained in the globulin fraction of the serum, and methods are now being devised for the concentration of the serum, so as to avoid the injection of a very large part of the serum protein which contains no immune substances. In this way it will probably be possible to avoid serum sickness, which has occurred in a number of our patients in from ten to ewelve days after the administration of the large amounts of horse-serum. This serum-sickness, while causing some discomfort to the patients, is not of any serious import, so far as we know.

It may be possible later to produce polyvalent serums that are efficacious. At present, however, and until the value of the special serum in the cases due to organisms of Groups 1 and 2 is unquestionably determined, it does not seem to be advisable to make such attempts. The objection is frequently raised that this method of treatment is very complicated. One may reply to this that so is the treatment of appendicitis.

At the present time I can do no more than mention the efforts along other lines that have been made to produce curative results by specific measures. Most important studies were made by the late Professor Hiss in the treatment of bacterial inflictions by means of leukocytic extracts. So far as concerns pneumonia, the results of experiments on animals are not very convincing, but the brief clinical report of cases of pneumonia treated, as stated in the article published since his death, seems extremely favorable and promising. It is to be hoped that study along this line will be continued.

Lamar has devised a method for the local treatment of pneumococcus infections. He has shown that immune serum has a much greater effect on pneumococci treated with sodium oleate solutions than on cocci simply washed in salt solution. This action of the soap, however, is inhibited if the serum be added first or mixed with the soap solution before treating the bacteria. The inhibiting action of serum, however, may be prevented by the addition of small amounts of boric acid, as Liebermann and von Fennyvessy have shown. By combining the soap, serum and boric acid in proper concentrations, Lamar has found a mixture that is much more efficacious in the local treatment of experimental pneumococcus infections than is serum alone. The treatment of local infections, as meningitis, with such a mixture, using serum effective against the race of organisms concerned, should be tried in all suitable cases. It is doubtful, however, whether such a mixture can be employed intravenously.

A final possible method which may be rendered practical in the treatment of pneumonia is along the lines of chemotherapy, as laid down by Ehrlich. It has been generally held that such a method of treatment may be of value in protozoan infections, but not in diseases due to bacteria. Morgenroth[42] and his coworkers, however, have shown that a derivative of quinin—ethylhydrocuprein—has a specific action on pneumococcus infections in mice, and Wright has shown that this drug is bactericidal for pneumococci in the test-tube. The drug has been employed clinically, but cases of amblyopia developing have indicated that the toxic dose in man too closely approaches the curative dose to permit the safe administration of the drug. It is possible, however, that with further study, its toxic properties may be reduced without lessening its curative effect.

CONCLUSIONS

Much obscurity still exists concerning the mode of natural infection in pneumonia, though by animal experimentation many facts in regard to it have been discovered.

The symptoms in pneumonia are probably due to toxic substances derived from the bacterial cells.

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41. Morgenroth: Berl. klin. Wchnschr., 1911, No. 44.

The outcome is dependent on the virulence of the organisms concerned and on the ability of the body, first to limit the local infection, and second, to prevent the invasion of the blood by the organisms, as on the latter the outcome of the disease mainly depends.

Leukocytes probably play a part in the resistance, certainly as regards the local spread and probably also to some extent as regards the general infection.

The most important part in prevention of the general infection is probably played by immune substances contained in the serum. Such substances are present in the serum of immunized animals.

Pneumococci differ in regard to their immunological reactions and on these they may be divided into several groups.

In order to use immune serum effectively in treatment, as in prevention, it is necessary to employ the serum effective against the group of organisms to which the special organism causing the infection belongs.

Immune serums effective against two of the most important groups have been produced. This treatment has been carried out in a limited number of patients with promising results.

It is probable that the methods of application of such serums will be improved, and it is possible that the method may be combined with other measures directed toward other factors which are important for the outcome. In any case, facts regarding the nature of the disease are being disclosed, and the outlook, at least for lessening the ravages of this dreadful disease, is encouraging.

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THE COLOR-INDEX OF THE RED BLOOD-CORPUSCLES *

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At first sight it may seem strange that it should be necessary at this date to call attention to some of the earliest observations in clinical hematology. Nevertheless, it is not difficult to show that then, and even now, the principles underlying these observations have not always been kept clearly in mind.

It will be remembered that Hayem[1] called attention to the disproportionately high colorimetric values of the blood in pernicious anemia as compared with the colorimetric power of the same number of normal red blood-corpuscles. Quincke[2] noted an apparent increase in the average hemoglobin content of the red blood-corpuscles in pernicious anemia. Later Laache[3] made a systematic investigation of the hemoglobin content and of the number of erythrocytes of the blood in health and in disease, and defined precisely what we now call the color-index of the red blood-corpuscles. Laache obtained his values for the color-index by taking thirty men and thirty women in apparent good health and establishing for each set the average hemoglobin content and the average number of erythrocytes. The ratio of the averages, separately for the men and the women, was taken as 1. From this were derived all of the individual indices of that series and of all subsequent determinations with the same hemoglobinometer and in terms of the same counting apparatus. Determined in this way, the color-index becomes independent of any constant methodical error in either determination, provided that the dilutions and concentrations are really proportional to the scales of the measuring device employed.[4]

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* From the Second Medical Clinic of the University in Munich, Prof. Friedr. Müller, director.

* Aided by a grant from the Rockefeller Institute for Medical Research, New York.

[1] Hayem: Du Sang, Paris, 1889. (Reference to first observations).

[2] Quincke: Deutsch. Arch. f. klin. Med., 1880, xxv, 577.

[3] Laache: Die Anämie, Christiania, 1883.

[4] This was tested by Laache for the Malassez hemoglobinometer. Although he used different methods for enumerating the red blood-corpuscles, he reduced all of his figures to the terms of one method, having previously determined the differences between the several methods. From the apparent differences he concludes that his results on the enumeration of the red blood-corpuscles were only relative.

For the color-index determined by Laache, the extreme variations for normal blood were from 0.9 to 1.1. In pernicious anemia the index reached in one case 2.3, while in chlorosis it was sometimes as low as 0.4. After this work of Laache, the value of the high color-index as a diagnostic sign of pernicious anemia became generally accepted. It is extremely rare, if ever, that a high color-index determined according to the principles of Laache has been found in other diseases. Erich Meyer and Albert Heineke[5] observed a high color-index in normal fetal blood at 5 and 7 months. This observation was important for the interpretation of the blood-picture and histologic findings in pernicious anemia. W. Türk[6] was inclined to regard the results of Meyer and Heineke as being due to a methodical error in the hemoglobin determination. In their reply to Türk, Meyer and Heineke[7] showed that they followed the principle of Laache, which frees their results from any constant error of the methods.

In spite of Laache's work, the question of the color-index has arisen now and again, chiefly because most of the clinical methods of hemoglobin determination are purely relative and many of them highly unsatisfactory. It seemed desirable, therefore, to go over this field again with exact methods, and establish the absolute values for the total hemoglobin concentration and the number of erythrocytes of the blood in health and in disease. As our real aim was not only to establish beyond any remaining doubt the existence of a high color-index in pernicious anemia, but also to ascertain the nature of the phenomenon, it was first necessary to study separately the hemoglobin of anemic blood, particularly with reference to its iron content. oxygen content and light absorption. In 1908-1909 it was established in Tübingen that the light absorption, oxygen content, and iron content of the oxyhemoglobin of the blood in pernicious anemia, chlorosis and polycythemia was identical with that of the oxyhemoglobin in normal human blood.[8] This proved the constant composition of oxyhemoglobin in health and in the essential blood-diseases, at least as far as the significant factors, spectrum (color), oxygen absorption (function), iron content and molecular weight were concerned. After this had been established the question of the absolute values for the hemoglobin content and number of erythrocytes of the blood in health and disease was taken up in Munich in 1909. The results of these determinations, as well as the values for the color-index calculated therefrom, are given in the following pages.

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[5] Meyer, Erich, and Heineke, A.: München. med. Wchnschr., 1906, liii, 793.
[6] Türk, W.: München. med. Wchnschr., 1907, liv, 220.
[7] Meyer, E., and Heineke, A.: München. med. Wchnschr., 1907, liv, 327.
[8] Butterfield: Ztschr. f. physiol. Chem., 1909, lxii, 173.

METHODS

The blood was taken in all cases from a vein of the arm, immediately defibrinated, and strained through gauze. Before each further step, the enumeration of erythrocytes, hemoglobin determinations, etc., the defibrinated blood was thoroughly shaken and continuously stirred while the pipettes were being filled.

Enumeration of Erythrocytes.—A Thoma-Leitz counting-chamber and pipette were used for all counts. The depth of the counting-chamber was determined micrometrically with the microscope, first with air, then with liquids of different refractive indices between the cover-glass and counting surface. The average of forty-five measurements of the distance between the under surface of the cover glass and the surface of the counting-chamber was 0.10 mm. ± 0.002 mm., provided interference bands of the third order were present throughout the film of water between the cover-glass and underlying glass plate. No counts were made unless these interference bands were present on all sides of the preparation. The total volume of the pipette was determined by weighing it empty and then filled with water. The volume of the capillary portion at the marks 0.5 and 1.0 was determined by weighing out with mercury. Each calibration was made in duplicate. The dilution, using 0.5 of the capillary content, was 186 instead of 200, and on using 1.0 of the capillary content, it was 101 instead of 100. Hayem's solution was used to prepare the dilutions for the counts.

Spectrophotometric Determination of Hemoglobin.—For the determination of the oxyhemoglobin content of the blood, the spectrophotometer of König, Martens and Grünbaum[9] was used with a white light (Nernst lamp) as a source of illumination. The spectrophotometric method is based on an empirical law, the so-called law of Lambert and Beer. When a bundle of parallel rays of monochromatic light enters under rectangular incidence and traverses a plane-parallel layer of a transparent homogeneous substance, the intensity of the bundle undergoes a certain diminution, apart from surface reflection. The amount of diminution depends on the nature of the substance, the wave-length of the monochromatic light and the concentration and linear thickness of the absorbing layer. The law of Lambert and Beer expresses the mathematical relation existing between the initial and final intensities of the monochromatic light in the solution and the concentration and linear thickness of the absorbing layer of the solution. The usual form is:

$$I' = Ie^{-klc}$$

in which I = the initial intensity, I' = the final (emergent) intensity in the solution, k = a constant which depends on the substance and on the wave-length of the light, l = the linear thickness of the absorbing layer, and c = the concentration of the solution. $\frac{I}{I'}$ is the quantity which is capable of measurement with the spectrophotometer. If k has been previously determined for the pure substance and l is known, then it is obvious that the concentration of the solution c, can be readily calculated when $\frac{I}{I'}$ has been measured. As a matter of fact k was determined with solutions of oxyhemoglobin from ox blood, after it had been previously shown in Tübingen with the Hüfner spectrophotometer that the optical constants of oxyhemoglobin from ox-blood and from human blood were practically identical in two regions in the spectrum.[10] The linear thickness of the absorbing layer was in all cases 2 cm. Strictly speaking, the fundamental equation holds only for monochromatic light, that is, light of a single wavelength. In the present case, however, the intensity ratio was measured over a small series of wave-lengths comprising about 4 μ. This is not accompanied by an appreciable error as long as the concentration and linear thickness are varied within narrow limits.

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[9] Martens and Grünbaum: Ann. d. Physik., 1903, iv, Series 4, 984.

[10] Butterfield: Ztschr. f. physiol. Chem., 1909, lxii, 194.

The measurements were always made at two regions in the spectrum, (1) in green, at the maximum of the absorption band of oxyhemoglobin and (2), in yellow, at the minimum of absorption between the two absorption bands of oxyhemoglobin. The advantage of this procedure becomes apparent on combining the equations for the drop in the light intensities at two different wave-lengths for the same solution in the same linear thickness. Let the relations at one wave-length be represented by:

$$I_1' = I_1 e^{-k_1 lc}$$

and the corresponding relations at the other wave-length by:

$$I_2' = I_2 e^{-k_2 lc}$$

Then:

$$\frac{\log \dfrac{I_1}{I_1'}}{\log \dfrac{I_2}{I_2'}} = \frac{k_1}{k_2} = \mathrm{K}$$

This affords a convenient and ready means of detecting variations in the spectrum of the blood under examination. It is possible to detect the presence of other derivatives of hemoglobin in the presence of oxyhemoglobin, which is of importance for the study of the probable nature of the color-index. The indices sub-one and sub-two denote in this paper a small spectral region at the maximum absorption of oxyhemoglobin in green and at the minimum in yellow respectively, k_1 and k_2 are the constants of oxyhemoglobin from ox-blood in these regions and have the values 19.4 and 11.7. Either one may be used for the determination of total hemoglobin concentration of the blood. In order to avoid confusion with other studies in which monochromatic light was employed and the same notation used,[11] it may be well to state again that in the following pages

$$\log \frac{I_1}{I_1} = \log \frac{I}{I'} \text{ at the maximum of absorption in green, and}$$

$$\log \frac{I_2}{I_2'} = \log \frac{I}{I'} \text{ at the minimum in yellow.}$$

For the spectrophotometric measurements the blood was laked in all cases with distilled water. The dilutions were prepared by emptying the contents of an Ostwald pyknometer into volumetric flasks.

Under the conditions of the measurements, the light absorption of the serum is negligible, as was first established by Vierordt. This point has been repeatedly checked with normal and pathological serums in a 2 cm. layer.

Hemoglobin Determination by the Sahli Method.—In most cases determinations were also made with the Sahli apparatus. In such cases the final reading was not taken until the maximum browning of the aqueous blood and hydrochloric acid mixture had been reached. This sometimes required twenty-four hours.

Specific Gravity.—The specific gravity was determined with an Ostwald pyknometer, weighing first when empty, then when filled with blood, and finally, with distilled water at a known temperature.

The accompanying table gives the results in full.

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[11] Butterfield: Proc. Soc. Exper. Med. and Biol., 1913, x, 74; Butterfield and Peabody: Jour. Exper. Med., 1913, xvii, 587.

It will be seen at once that the extreme variation for the color-index of normal blood in our cases is from 0.9 to 1.1. In pernicious anemia the index is markedly raised, as is also the average hemoglobin content of the red blood-corpuscles. In secondary anemia a low index is found. Unfortunately, chlorosis was not included in the present series. In all probability, the values here presented for pernicious anemia and secondary anemia do not represent the extreme possible variations.

TABLE 1.—Hemoglobin Determinations in Blood from Normal and from Pathologic Subjects

NORMAL BLOOD

	Specific Gravity	Sahli Reading	Erythrocytes Millions per cu.mm.	$\frac{\text{Log}\frac{I_1}{I'_1}}{\text{Log}\frac{I_2}{I'_2}}$	Hemoglobin Content of Blood gm. per 100 c.c.	Quantity of Hemoglobin in Average Erythrocyte, gm. $\times 10^{-11}$	Color Index
Men, 1	1.057	95	4.29	1.66	15.4	3.6	1.1
2	1.055	77	4.19	1.65	14.3	3.4	1.0
3	1.062	100	5.19	1.67	17.4	3.2	0.9
4	1.061	97	5.46	1.68	17.8	3.3	1.0
5	1.056	87	4.57	1.64	15.3	3.4	1.0
6	1.066	105	5.79	1.65	19.3	3.3	1.0
7	1.060	93	4.74	1.65	16.9	3.6	1.1
Averages	1.059	93	4.92	1.66	16.6	3.4	1.0
Women, 1	1.057	83	4.42	1.64	15.8	3.6	1.1
2	1.058	88	5.11	1.64	15.6	3.1	1.0
3	1.053	77	4.44	1.67	13.7	3.1	1.0
4	1.061	97	5.11	1.64	17.0	3.3	1.0
5	1.055	85	4.46	1.64	13.7	3.1	1.0
6	1.056	90	4.95	1.65	15.2	3.1	1.0
Averages	1.057	87	4.75	1.65	15.2	3.2	1.0

PATHOLOGICAL CASES

	Specific Gravity	Sahli Reading	Erythrocytes Millions per cu.mm.	$\frac{\text{Log}\frac{I_1}{I'_1}}{\text{Log}\frac{I_2}{I'_2}}$	Hemoglobin Content of Blood gm. per 100 c.c.	Quantity of Hemoglobin in Average Erythrocyte, gm. $\times 10^{-11}$	Color Index
Pernicious anemia I	1.040	21	0.74	1.65	3.47	4.7	1.5
" " II	1.035	23	0.87	1.65	3.79	4.4	1.3
Secondary anemia..	—	—	2.43	1.63	5.59	2.3	0.7
Polycythemia	1.075	—	—	1.66	23.9	—	—

From the general agreement with Laache's results, however, there seems to be no reason for hesitating to accept his extreme values of 0.4 to 2.3.

Passing to the absolute values, it will be noticed that the average hemoglobin content of normal blood is considerably higher than the accepted value of 14 gm. in 100 c.c. of blood. There are two reasons for this. In the first place, defibrinated blood was used in all cases in the present work, and it is possible that there is a slight decrease in volume after removal of the fibrin. In the second place, the old value of 14 gm. in 100 c.c. dates back chiefly to Otto's[12] determinations with the old model of the Hüfner spectrophotometer, using the optical constant of oxyhemoglobin from dog's blood. It is not permissible to employ the constant of dog hemoglobin for determinations on human blood, unless one has previously established the identity of the optical constants for both sorts of hemoglobin. As has been mentioned, this was done in the case of oxyhemoglobin from human blood and from ox-blood. If the determination of the constants has been carried out with the proper precautions, it would seem to be immaterial what spectrophotometer is used, provided, of course, that the construction and adjustment are correct. For example, we found in Tübingen with a new model of the Hüfner spectrophotometer, a hemoglobin content of 16.3 gm. in 100 c.c. of the mixed defibrinated blood from three healthy men, a value which is in close agreement with the average for men in Munich, determined with spectrophotometer of König, Martens and Grünbaum, that is, 16.6 gm. in 100 c.c.

Especial emphasis is to be laid on the constancy of

$$\frac{\log \dfrac{I_1}{I_1'}}{\log \dfrac{I_2}{I_2'}}$$

for normal human blood and for the blood in pernicious anemia, polycythemia and secondary anemia, which goes to prove that in these conditions there is no constitutive change in the oxyhemoglobin molecule which can be detected optically. From previous work it also follows that little or no other change is probable, inasmuch as the oxygen capacity and the iron content are also constant per gram hemoglobin in the conditions mentioned and in chlorosis.

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[12] Otto: Arch. f. Physiol. (Pflüger's), 1885, xxxvi, 36.

NATURE OF THE HIGH COLOR-INDEX

Since the color-index was first observed, up to the present time, various explanations have been advanced. It was first suggested that the high color-index was only apparent — that a number of small erythrocytes were either broken up and dissolved in the diluting fluid or missed in the counting. Laache was inclined to believe that in some cases the high color-index could be accounted for by the number of macrocytes. In fact, in going over his figures one finds in some of his cases that the color-index is almost proportional to the square of the average radius of the erythrocytes. The same is true in the work of Capps[13] on the volume-index, which he found to follow closely the color-index. This explanation by the increase in the total volume of the erythrocytes seems to be the most probable. It is really immaterial for the present, however, whether the phenomenon is due solely to an actual increase in the volume of the erythrocytes with constant intracellular hemoglobin concentration, or at the same time partially due to an increase in the hemoglobin concentration of some of the erythrocytes. The question of primary importance may be stated in the following terms. Given a blood with a high color-index and one with a low color-index, but with the same total hemoglobin concentration: Does the distribution of the same quantity of hemoglobin over a different number of particles, that is, red blood-corpuscles, affect the oxygen capacity of the native blood? This can be settled only with suspensions of red blood-corpuscles from the essential blood-diseases, and almost all of the previous work has been done with laked blood.[14]

Recently, Pappenheim[15] has advanced the view that the high color-index is due to a true *hyperchromatism* of the erythrocytes. He does not regard this in the light of an increase in the hemoglobin concentration within the erythrocyte, but assumes a degeneration (?) of the blood coloring-matter with the formation of methemoglobin or promethemoglobin (?). There is absolutely no evidence in support of such a view as far as pernicious anemia of human pathology is concerned.[16] In fact, there is conclusive evidence against this view. We mentioned in the beginning that the oxyhemoglobin in pernicious anemia is identical with that of normal blood, as far as the light absorption, gas absorption and iron content are concerned. That the high color-index should be caused by methemoglobin is contrary to actual facts. With the exception of the band in red, methemoglobin in neutral solution absorbs in yellow, green and violet less light than oxyhemoglobin.[11] When the colorimetric power of the blood is determined, as hematin with the Sahli apparatus, it is possible that methemoglobin may give an apparently higher reading than oxyhemoglobin, but only when the reading is made shortly after adding the hydrochloric acid and diluting. If the determination is made properly, that is, the reading taken after awaiting the maximum browning of the solution, the presence of methemoglobin should not influence the Sahli method. As far as direct measurements of the light absorption are concerned, the presence of methemoglobin would give an apparent decrease in the oxyhemoglobin content, if the measurements were made in yellow, green or violet; exactly the opposite of Pappenheim's contention.

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[13] Capps: Jour. Med. Research, 1903-1904, v, 367.

[14] Experiments with suspensions of red blood-corpuscles are in progress. The method of Paal, which consists in reduction with colloidal palladium in an atmosphere of hydrogen, is well suited to this work. Experiments with normal red blood-corpuscles suspended in salt solution have already been carried out. It has been found that the oxygen content of suspensions of normal red blood-corpuscles is proportional to the total hemoglobin concentration. Two suspensions have been used. One contained red blood-corpuscles corresponding in number to normal blood, while the other was ten times more dilute.—*Butterfield.*

[15] Pappenheim: Fol. Haematol., 1911-1912, xii, Part 2, p. 68.

THE COLOR OF THE RED BLOOD-CORPUSCLES

The study of the light absorption of oxyhemoglobin leads naturally to a discussion of the color of the red blood-corpuscles. It is well known that single corpuscles viewed in transmitted daylight are light green or yellowish green in color. The red tinge does not become noticeable until transmitted light traverses three or four superposed corpuscles. As far as we know, a satisfactory explanation of this phenomenon has never been advanced. A comparison of the spectra of the greenish single layer and of the reddish superposed layers gives, we believe, the clue to the explanation of the phenomenon. It will be observed that there are two minima of absorption, that is, two maxima of intensity of the transmitted light, one in red and the other in yellowish green. On increasing the thickness of the absorbing layer, that is, the number of superposed corpuscles, the intensity of the transmitted light at the absorption minimum in yellowish green diminishes abruptly, while the diminution of intensity in red is inappreciable. This means that the constant of light absorption at the minimum in green is much greater than the corresponding constant at the minimum in red, in symbols $k_{gr} > k_r$. If we regard the intensities in these two regions only, assuming that the absorption in the other regions of the spectrum is so great that the transmitted intensities are negligible,[17] then $I_{gr}e^{-k_{gr}lc} + I_re^{-k_rlc}$ would represent the intensity of the transmitted light.

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[16] Pappenheim[15] bases this view on the assumption that the chemicals which are used for the production of experimenttal anemia are at the same time substances which convert oxyhemoglobin into methemoglobin. As far as we are aware, however, a high color-index in the sense of Laache has never been observed in experimental anemia, toxic or posthemorrhagic. His "proof" of his view is as follows: 1. One determines the coloring-power of a portion of centrifuged and washed sheep corpuscles by the Sahli method. 2. To an equal portion of the washed corpuscles is added hydroxylamin, pyrodin, or toluylenediamin dissolved in a drop of physiological salt solution or distilled water. The coloring-power is then determined by the Sahli method. It will be found that the Sahli reading in the second case is almost double that of the first. This is not at all remarkable when one considers that the Sahli method rests on the conversion of oxyhemoglobin into hematin and subsequent comparison with a "standard" hematin solution. The conversion of oxyhemoglobin to hematin requires time, especially with the Sahli technic (tenth-normal hydrochloric acid at room temperature). It will be found that if one takes a Sahli reading immediately after adding hydrochloric acid and diluting with water up to apparent equality with the "standard" solution, and then lets the preparation stand twenty-four hours, it will often be necessary to dilute further, so that the final reading is often 20 per cent. higher than the initial reading. On the other hand, if methemoglobin is present, the brown color of methemoglobin is much nearer that of hematin, so that in the gradual conversion to hematin, the maximum browning may be reached sooner than in starting from pure oxyhemoglobin. In all cases before complete conversion has been accomplished, one is comparing a mixture of oxyhemoglobin, methemoglobin, and hematin with the "standard" hematin solution. Hence the confusion. Taking the proper precautions, one of us (*B.*) has repeated the Pappenheim experiment with a completely negative result.

Starting from a number of superposed corpuscles which appear red in transmitted light and decreasing progressively the number of corpuscles superposed, one would expect, in accordance with the principle of dichromatism,[18] that a color change from red to green would occur if the condition, $I_{gr}e^{-k_{gr}lc}$ equal to or greater than $I_re^{-k_rlc}$, could be realized. In the case of red blood-corpuscles viewed in transmitted daylight the necessary conditions, $I_{gr} > I_r$, $k_{gr} > k_r$, and l sufficiently small, are all favorable to the realization of $I_{gr}e^{-k_{gr}lc} > I_re^{-k_rlc}$. It would be reasoning in a circle to say that this is a demonstration of the cause of the green color of a single layer of red blood-corpuscles. Even if it were found that for the correct values of I_{gr}, I_r, k_{gr}, and k_r, the observed l, at which the color change occurred, agreed with the calculated, then the demonstration would not be complete, for one must account for the sum of all intensities in the transmitted light, and this leads to difficulties, mathematical and experimental, which are at present insurmountable.

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[17] Strictly speaking, one should formulate for the intensities over the whole visible spectrum. This leads to an integral which has no practical significance as long as we do not know what functions I and k are of λ (the wave-length). See remarks of S. P. Langley: Am. Jour. Sci., 1884, Third Series, xxviii, 168.

[18] Dichromatism must not be confused with dichroism. Dichromatism depends on the selective absorption of isotropic substances, while dichroism is a special case of pleochroism, which refers to anisotropic crystals in which the light absorption varies with the direction of the vibrations of the light waves.

There remains, however, the possibility of a demonstration by exclusion. In the first place, it is possible to exclude the effect of the stroma and of the surface curvature on the color of the red blood-corpuscles. A very thin wedge of solid oxyhemoglobin can be made by allowing a drop of a concentrated solution of oxyhemoglobin to evaporate under a cover-glass 6 cm. long, of which one end is very slightly raised. The linear thickness of different parts of such a wedge can be estimated from the interference phenomena which are visible in the spaces unoccupied by oxyhemoglobin, between the cover-glass and slide. At 0.2 microns the oxyhemoglobin is colorless, between 0.2 microns and 1.3 microns the oxyhemoglobin is yellowish green, that is, of the same color as red blood-corpuscles in a single layer, while above 1.3 microns the reddish tinge is noticeable and increases with the thickness of the wedge to a vivid red. The color change is the same with illumination by a bundle of parallel rays and with illumination by bundles with different degrees of convergence. This, again, is not a rigid proof of the dichromatism of red blood-corpuscles, but it does prove that the color change observed with red blood-corpuscles may be perfectly imitated with a homogeneous isotropic layer of oxyhemoglobin of varying thickness.[19] This, taken in conjunction with the spectroscopic appearances, eliminates dichroism, surface reflection and interference as necessary factors for the explanation of the color of the red blood-corpuscles, and leaves by exclusion the dichromatism of oxyhemoglobin as the most probable explanation of the phenomenon.

CONCLUSIONS

1. The existence of a high color-index in pernicious anemia has been established with exact methods. The high color-index is due to an increase in the oxyhemoglobin content of the erythrocytes. A quantitative study of the light absorption shows that oxyhemoglobin alone and no other derivative of hemoglobin is present in the blood of cases of pernicious anemia with a high color-index.

2. The color-index of normal human blood varies within the narrow limits of 0.9 to 1.1. The average total hemoglobin concentration of normal defibrinated human blood is in the neighborhood of 16 gm. in 100 c.c. The color of the red blood-corpuscles is best explained by the principle of dichromatism as applied to the absorption spectrum of oxyhemoglobin.

Bellevue and Allied Hospitals.

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[19] It may be mentioned, incidentally, that very small (microscopic) crystals of oxyhemoglobin seem to be identical in color with the red blood-corpuscles, while larger crystals are bright red.

HUMAN STREPTOTRICHOSIS AND ITS DIFFERENTIATION FROM TUBERCULOSIS *

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Members of the somewhat ill-defined group of micro-organisms, *Streptothrix,* produce the disease streptotrichosis in man and animals. There is a gradually increasing recognition of the close relationship between these organisms and the bacillus of tuberculosis; this, together with an equal similarity between these two diseases, focuses our interest sharply on the problem of their prompt recognition and early differentiation from each other.

The literature of the subject is extensive, and to be found chiefly in periodicals. A few of the large handbooks of medicine, Osler's and Allbutt's, give clear discussion of the mold fungi, as to their structure and as agents in disease production; but on the whole the usual medical text-books pass the subject casually, so that the student of medicine has no very good idea of the biological characters of, and relations between the streptotriches, sporotriches and blastomycetes, or of their really important rôle in the production of disease in man and animals.

The observations of these fungi made by Mayen, 1827; Corda, 1842; Langenbeck, 1845, and others are our earliest records. Carter from 1859 to 1874 first clearly proved the pathogenic rôle of streptotriches in man, his work centering on Madura foot. The classic work of Bollinger[1] (1877), Hartz[2] (1878), and Israel[3] (1878) established the recognition of the disease and its cause in both man and cattle. Boström[4] (1891) completed an exhaustive laboratory study of the organisms isolated from eleven cases of streptotrichosis. Wolff and Israel did likewise in the same year. Many others have added to our knowledge of these fungi and their lesions, the greater part of the published material being, however, more or less complete case reports

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[1] Bollinger, O.: Ueber eine neue Pilzkrankheit beim Rinde, Centralbl. f. med. Wissensch., 1877, xv, 481.

[2] Harz, C. O.: Actinomyces bovis, ein neurer Schimmel in den Geweben des Rindes, Jahresb. d. k. Centr. Thierarzneischule in München, 1877-8, p. 125.

[3] Israel, J.: Neue Beobachtungen auf dem Gebiete der Mykosen des Menschen, Virchows Arch. f. path. Anat., 1878, lxxiv, 15.

[4] Boström: Demonstration mikroskopischer Präparate von Aktinomykose der Menschen und Thiere, Berl. klin. Wchnschr., 1885, xxii, 14, 274.

with various cultural data, some purely biological and some including experimental studies, with a few articles giving a well-balanced critique of the whole subject. The last-mentioned serve to show how much a provisional basis of classification and a working hypothesis are needed for this group, so that observations may be comparative. In recent years Vincent,[5] Wright[6] and especially Foulerton[7] have done much to clear away the confusion surrounding these organisms. Musgrave, Clegg and Polk[8] offer a bibliography of over 1,500 references as well as a good historical summary, a critical biological study of six species and some significant experimental data on monkeys. Their work had especial reference to the various forms of Madura foot.

In this mass of facts, clinical, biological and pathological, there are several that stand out prominently. The appearance of the disease is in distinct types; as a rule slow, progressive, markedly local, but capable of an acute, rapid general pyemic or pneumonic course, with a fatal termination in from one to three weeks. These cases may be especially confusing, since little or no discharge is present in which to find the organisms, and probably these are in a bacillary or rod-segment stage, not the fungoid threads and granules usually encountered and looked for. A slow chronic case may thus flare up and terminate fatally; especially is this noted in secondary cerebral involvement following lung or bone infection, making a complete analogy to tuberculous meningitis.

The confusion between tuberculosis and streptotrichosis is well illustrated by many writers in their reports on pseudotuberculosis and pseudo-actinomycosis. The rôle played by acid-fast and bacillary streptotriches is here prominent, still further masking the nature of the infection and so allying it apparently to tuberculosis. The use of carbolfuchsin as a stain is a further cause for confusion, since usually only the acid-fast parts of the streptothrix are thus demonstrated, while with the Gram method both the acid-fast and non-acid-fast elements stain equally well. One of the earlier descriptions of these acid-fast types is Nocard's[9] original discussion of *Streptothrix*

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[5] Vincent, H.: Etude sur le parasite du "pied de Madura," Ann. de l'Inst. Pasteur, 1894, viii, 129.

[6] Wright, J. H.: The Biology of the Micro-Organism of Actinomycosis, Jour. Med. Research, 1904, xiii, 349.

[7] Foulerton, A. F. G.: Streptothrix Infections, Lancet, London, 1899, ii, 779; Pathology of Streptothrix Infections, ibid., 1906, i, 970; Streptotrichosis and Its Relation to Tuberculosis, ibid., 1910, i, 551, 626, 771; Some Observations on a Series of Seventy-Eight Cases of Streptothrix Infections, ibid., 1913, i, 381.

[8] Musgrave, Clegg and Polk: Streptotrichosis, with Especial Reference to the Etiology and Classification of Mycetoma, 1903, iii, 447.

[9] Nocard: Note sur la maladie des bœufs de la Guadeloupe, Ann. de l'Inst. Pasteur, 1888, ii, 293.

nocardii (*farcinica*), an acid-fast strain that causes *farcin du boeuf*, a disease suspected of being tuberculosis until Nocard's clear demonstration that the organism was an acid-fast streptothrix. In laboratory cultures the acid-fastness is but partial; only certain of the individual rods remain red, the rest taking the counter-stain. In 1898 Flexner[10] discussed under the name "pseudotuberculosis" a case of a man aged 70 with both lungs extensively consolidated and the clinical symptoms of tuberculosis. No sputum was obtained, the diagnosis being made post mortem. No tubercle bacilli, but faintly red-staining, branched organisms with no bacillary forms, were found in smears of lung, and a slightly different form, more bacillary, from peritoneal nodules. Cultures showed no growth. An article in the same year by Berestneff[11] on pseudo-actinomycosis drew attention to the similarity of its pathology to that of actinomycosis and the absence of the granules and clubs then supposed to be essential to actinomycotic infection. Lubarsch[12] saw the relationship of *Bacillus tuberculosis* and its nearly related acid-fast neighbors to the acid-fast and non-acid-fast streptotriches, and the similarity of the diseases which they produce.

Feistmantel,[13] after careful experiment and critical observation of *Streptothrix nocardii* (*farcinica*) as to its acid-fastness and variations in structure in tissues, asserted its close relation to the acid-fast organisms on the one side and actinomyces on the other, calling them all *Streptothrix*. Bert and Leishmann[14] reported a pulmonary infection of a dragoon at Ladysmith, South Africa, with an acid-fast streptothrix showing branched and unbranched rods. The organism in pure culture was obtained from the lung and pericardial fluid at necropsy. In the same year Ophüls[15] reported on five cases of pulmonary gangrene in California caused by acid-fast, branched bacilli from which pure cultures were not obtained. He considers that the bacteria belong probably to the streptothrix group associated with pyogenic cocci in causing the disease. Sanfelice[16] and Galli-Vallerio[17] have also given further evidence as to the pseudotuberculoses caused by streptotriches, and the close relationship between them and the whole group of acid-fast organisms. This property of acid-fastness is, however, a variable one; it may be considered a function rather than a structure, since it can be assumed or discarded by the organisms apparently according to circumstances; for example, the butter bacillus gradually assumes acid-fastness when lying in the fat, losing it again when removed to a fatless medium. Sanfelice suggests that the oil in the skin of those parts of the body normally associated with acid-fast organisms assists in developing this property. Some remarkable work has been done in the last few years on the organism of leprosy. Williams[18] has isolated from leprous nodules three types, a non-acid-fast mycelium and non-acid-fast diphtheroid organism, and an acid-fast bacillary and mycelial form; both the former under appropriate conditions develop acid-fast properties, the diphtheroid forms doing so in forty-eight hours when devoured by amebas; those simply lying near the protozoa may develop some acid-fast granules. The acid-fast rods disappeared after the third subculture, and with appropriate subcultures the organisms again assumed acid-fastness. These observations have been corroborated by other writers. Williams used a vaccine made from the acid-fast cultures, which called out an apparent specific reaction on injection into lepers. The non-acid-fast type failed to do this, but from past experience in serum reactions it seemed necessary to use specially efficient endotoxin extraction (Besredka's, Gay's modification) for antigens in the mycelial types. Reactions would probably occur if such preparations were used. The remarkable pleomorphism of this group of organisms is well illustrated in these results, and the great possibility of confusion and misapprehension as to the character of a strain isolated from any given infection.

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[10] Flexner, S.: Pseudo-Tuberculosis Hominis Streptothrica, Jour. Exper. Med., 1898, iii, 435.

[11] Berestneff, N.: Ueber Pseudo-Aktinomykose, Ztschr. f. Hyg., 1898, xxix, 94.

[12] Lubarsch: Zur Kenntniss der Strahlenpilz, Ztschr. f. Hyg., 1899, xxxi, 185.

[13] Feistmantel: Saure- und Alkohol-Festigkeit der Streptothrix farcinica und die Beziehungen der Streptotricheen zu den Saurefesten Pilzen, Centralbl. f. Bakteriol., Orig., 1902, xxxi, 433.

[14] Bert and Leishmann: A New Acid-Fast Streptothrix Pathogenic to Man and Animals, Jour. Hyg., 1902, ii, 120.

[15] Ophüls: Acid-Proof Bacilli in Five Cases of Pulmonary Gangrene, Jour. Med. Research, 1902, viii, 242.

[16] Sanfelice: Streptothrix-Pseudotuberculose, Centralbl. f. Bakteriol., Orig., 1905, xxxviii, 30.

[17] Galli-Vallerio: Etudes sur les Actinomycetes, Centralbl. f. Bakteriol., Orig., 1912, lxiii, 555.

The susceptibility and frequent infection of cattle is worthy of consideration; in fact, better discussions of this disease and its causal agents can be found in veterinary than in human text-books on pathology. Acute infections occur as well as the classic lumpy jaw. Kieffer[19] reports an outbreak of bovine pneumonia in a small dairy, five cows dying in a few days. A freely growing streptothrix was isolated from the blood and all viscera examined. Usually these bovine infections are caused by the well-recognized *Streptothrix bovis*, but the relation, if any, between the organisms in the slow chronic cases and rapidly fatal pneumonias is not established. With pleomorphic organisms, such as these streptotriches, it is to be borne in

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[18] Williams, T. S. B.: Leprosy: A New View of Its Bacteriology and Treatment, Brit. Med. Jour., 1911, ii, 1582.

[19] Kieffer, C. F.: A New Streptothrix Pathogenic to Cattle, Med. Rec., New York, 1907, lxxi, 346.

mind that a variation in pathogenicity due to changes in host, parasite or both can readily allow one morphological form to predominate. There is reason to think that the extension of the disease both in the diseased organ and from case to case is made by the rod segments or bacillary forms; they migrate, take root, as it were, forming a new plant which grows and casts off in turn these readily disseminated structures. It is a moot question whether streptotrichosis in man is caused by the same species of streptothrix which occurs in cattle. Foulerton, from his observations, believes the human cases to be only occasionally produced by *Streptothrix bovis,* some form of *Streptothrix hominis* or the partly acid-fast types being the usual agents. The necessity for a clear determination of specific characters and their limits of variability is emphatically demonstrated here.

There is an almost entire failure of early diagnosis in streptotrichosis. The impression gained from the case records is of an overwhelming mortality. Relatively few cures are recorded, largely because diagnosis is not made till a late stage, or even after death. In infections of the skin, it is more easily made early, and such cases afford the majority of cures. Early slight infections, perhaps spontaneously curable, pass unrecognized, and hence unrecorded.

There seems to be no marked geographical distribution of this disease, barring the tropical Madura foot, which, being essentially a wound infection, occurs particularly among the barefoot races. In Russia[20] there are reports of a large number of lung infections, whether owing to better diagnosis or greater incidence of the disease, or to both causes, cannot be determined. Opokin states that it is frequently undiagnosed or called tuberculosis. A series of such infections, chiefly of the lungs, was reported from California by Bridge.[21] They were found among a large number of presumably tuberculous cases. Captain Kremer, one of the Army medical staff stationed at Honolulu, speaks of the occurrences of these fungoid infections among the residents of the islands.[22] Whatever are the conditions, and they are probably complex, that lead to the frequency of the disease in the mild semitropical climate of the Hawaiian Islands, the moderate one of California, north and south, and the comparatively frigid Russia, this wide range shows the problem of these infections to be no small one. The frequent confusing clinical similarity to tuberculosis only adds emphasis to these facts. All clinicians are familiar with so-called tuberculous lung infections, without demonstrable bacilli, and it is

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20. Opokin, A. A.: Lungen- und Pluerenaktinomykose nach den Beobachtungen russischer Autoren, Arch. f. klin. Chir., 1909, lxxxviii, 319.

21. Bridge, Norman: Streptotrichosis (Actinomycosis) of the Lungs, Jour. Am. Med. Assn., 1911, lvii, 1501.

22. Kremer: Personal letter to author.

among such, especially, that cases of streptotrichosis are to be found.

The streptotriches, non-acid-fast, partly acid-fast, and wholly acid-fast, have long been associated with herbage quite as intimately as the well known acid-fast organisms allied to *B. tuberculosis*. Both saprophytic and pathogenic strains have been isolated from soil, water and foodstuffs. They are omnipresent. Petruschky[23] investigated the carpets in many rooms of an old public building in Danzig and demonstrated several strains near the windows; one strain was pathogenic to guinea-pigs. (This investigation followed the recognition of an infection in one of the men employed in the building.) Not only can they be found in an apparently saprophytic condition outside the body, but it is well known that certain apparently harmless strains can be demonstrated in the mouth and nasopharynx of man. Lord[24] has made some suggestive observations on the cheesy contents of the crypts of enlarged tonsils and also the material obtained from dental caries. In smears and in serial sections from both he obtained a typical Gram-positive streptothrix. Peritoneal inoculation of guinea-pigs yielded characteristic lesions with colonies of a branching organism in 60 per cent. of the animals examined at necropsy from twenty-one to forty-five days after injection. Lord believes that there is an etiological relation between it and the diseased teeth and enlarged tonsils. So-called non-pathogenic forms are frequently present in the mouth, particularly when any abnormal condition exists, as pyorrhea alveolaris, or otherwise abnormal teeth and gums.

With these facts in mind, data as to the location of streptotrichosis in the body assume greater significance. The percentage of cases occurring in the head and neck, as gathered from three divergent sources, is summarized in Table 1.

TABLE 1.—Head and Neck Infections with Streptotriches

	No. Cases	Per Cent. Cases
Foulerton (Middlesex hospital) London	78	51.0
Acland (British)	109	38.5
Ruhräh (American, collected cases)	632	57.0
Average		49.0

An average of 49 per cent., nearly half the recorded cases, involve the head and neck region, near which organisms with supposedly small, if any, pathogenic powers are frequently to be found. The slightly abnormal state of inflamed gums, enlarged tonsils, and decayed teeth, however, can readily offer the *locus minoris resistentiae* so essential to infection. Here also is emphasized the confusion possible with tuberculosis. There is nothing characteristic in the clinical appearance of abscesses or enlarged glands, or in fact of any form of streptotrichosis that differentiates it clearly. The recognition of the parasite in discharges is frequently difficult, and in many cases, especially early in the trouble, there is no discharge to examine. This is markedly so in cervical adenitis, usually assumed to be tuberculosis, in which only a careful examination of the removed glands in culture and section can determine the true nature of the disease in early stages. Burnet[25] reports an examination of twenty-three cases of cervical adenitis in children and adults that were clinically tuberculous; twenty-two yielded cultures of *B. tuberculosis* on Dorset's egg-medium; one a pure culture of a filamentous, branched organism, Gram-positive, non-acid-fast, producing red colonies, quite probably *Streptothrix rubra,* a species known to be at times pathogenic. It was obtained from the pulp of the gland. The latter was the size of a small egg, non-caseous, non-calcified with a consistence like a very firm carcinoma. A similar case came under my personal observation and will be reported on later.

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[23] Petruschky: Kolle und Wassermann's Mikroorganismen, 1912, v, 280, etc.

[24] Lord, F. T.: Actinomycosis: Its Production in Guinea-Pigs from Carious Teeth, Boston Med. and Surg. Jour., 1910, clxiii, 82; The Etiology of Actinomycosis, Jour. Am. Med. Assn., 1910, lv, 1261.

Records of chest infections are numerous, averaging about 18 per cent. of all cases of streptotrichosis. With greater accuracy of observation, this percentage would doubtless be increased. There are also a significant number of appendix infections, as Foulerton has especially pointed out. Of his seventy-eight cases twenty were of the appendix, with six deaths, due in four cases to suppurative thrombosis of the portal vein.

The need of some more ready and accurate means of diagnosis is evident. Clinical symptoms are not distinctive. The pathologist may make a diagnosis from discharges or tissues, examining them for gross granules or microscopic fragments by using the Gram stain, provided the lesions are not accepted as tuberculosis. Cultures are possible in some cases, but the well-recognized difficulty in the cultivation of some strains makes this method only partially successful. There remains the large and most important group of early cases without discharge and of those so deep seated as to be unavailable. The necessity of differentiation from tuberculosis is always present and rarely considered.

The results of a recent experimental study[26] of a series of these organisms and their serological reactions in immunized rabbits adds further evidence for the near relationship between the streptotriches and *B. tuberculosis.* The various partly acid-fast species are links connecting the mycelial, entirely non-acid-fast strains at one end with the entirely acid-fast *B. tuberculosis* and its allies at the other. The morphological variability and many of the cultural peculiarities common to these organisms and to the small but important group of the acid-fast bacilli also emphasize this community of stock. The mere fact of acid-fastness is no absolute criterion since there is experimental evidence (Williams,[18] Marino,[27]) to show that many species assume or discard it according to circumstances, the condition being somewhat analogous to the spore state in other bacteria, which offers more resistance to unfavorable surroundings. In the serological reactions referred to, the two end members of this series were clear cut from each other; while the intermediate members reacted with both extremes, the latter would not interact. It was this relation that suggested the preparation of glycerinated extracts, similar to Koch's old tuberculin, and their use in skin tests.

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[25] Burnet, E.: Streptothricee dans une adénopathie cervicale, Compt. rend. Soc. de biol., 1913, lxxiv, 674.

[26] Claypole, E. J.: Classification of the Streptotriches, Particularly in Their Relation to Bacteria, Jour. Exper. Med., 1913, xvii, 99.

STREPTOTRICHINS AND THEIR PREPARATION

Streptothrix hominis III (Foulerton) and *Streptothrix eppingeri* were selected, respectively, to represent the non-acid-fast mycelial and the partly acid-fast bacillary strains. The method of preparation of the streptotrichins is as follows:

Flasks of 5 per cent. glycerin bouillon were planted with the cultures and incubated at 37 C. In a week or two *Streptothrix eppingeri* began to form a floating, crinkled, bright orange membrane that heaped up in a manner similar to the characteristic growth of *B. tuberculosis*. This was allowed to grow for six weeks in Culture 1, and made streptothricin E. It is more difficult to make the *Streptothrix hominis* type float, but the addition of cork islands, on which the fragment of mycelium was originally placed, assisted materially in supporting a film. Culture 2 of this strain was grown for only two weeks, owing to the lack of successful film formation and the consequent drowning of the organism. From this, streptotrichin H was made. Preparations of the streptotrichins were made as follows, from Cultures 1 and 2:

They were heated one hour at 60 C., filtered through a porcelain filter and concentrated to one-tenth of their original volume, making 60 per cent. glycerinated extracts. These two streptotrichins, Koch's old tuberculin (O. T.) and a control of plain glycerin bouillon concentrated to one-tenth volume were used in the cases reported.

The skin test was performed in 100 cases, 55 abnormal persons and 45 controls, the latter presumably with no active disease of the sort under investigation; a few were convalescent from acute illness, some under surgical treatment, and 16 were children of from 3½ months to 11 years of age. Among the 45 controls, there were 22 tuberculin reactions and no streptotrichin reactions. These tuberculin reactions appeared in 17 adults and in 5 children, without clinical symptoms, but some with records of cases of tuberculosis in the family or old suspicious illness from which there was complete recovery. In the group of 55 abnormal cases, 36 positive and 1 doubtful tuberculin reactions were observed, 13 streptotrichin-H reactions, 8 streptotrichin-E reactions, and 6 double reactions. Forty-two of these cases had lung infections frankly tuberculous or only clinically so; 11 were gland cases, and 5 bone infections. Details of these cases and their reactions are given in Table 2. The skin tests were all made with a von Pirquet chisel, avoiding trauma as much as possible; then sterile wooden toothpicks were dipped into the respective preparations and the extract rubbed into the abrasions gently. They were allowed to dry for ten minutes, and examined at twenty-four- and forty-eight-hour intervals. There is a distinct difference between the reactions produced by the two streptotrichins. That of the party acid-fast streptotrichin-E is dense, indurated and long-lasting with an areola of from 6 to 10 mm., very similar to the typical tuberculin reaction, while the H-extract produces a more transient change very similar to that caused by the typhoidin used in the skin tests recently reported on by Gay and Force.[28] There is a characteristic areola from 6 to 10 mm. in diameter with a central induration that is quite perceptible to the touch. The reddened areola may be quite transient, disappearing in from twenty-four to forty-eight hours, while the induration may last several days, never, however, showing the dense nodosity so characteristic of the acid-fast reactions. It is apparent that the "tuberculonastin" and the fatty acids present in the tubercle bacillus or similar substances in the streptothrix produce a more intense local irritation. Such preparations have been shown by Deilmann[29] to cause more intense serum reactions than the non-acid-fast protein residue remaining after the extraction of these substances from the tubercle bacillus.

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[27] Marino, F.: Atténuation de la virulence du bacilli tuberculeux dans le tube digestif des hirudinées, Compt. Rend. Soc. de Biol., 1911, lxxi, 220.

STREPTOTRICHIN-H REACTIONS

Of the 13 streptotrichin-H reactions, 11 were in cases of pulmonary disease and 2 were glandular infections. Of the lung cases 6 were advanced, 2 early of moderate extent, and 2 patients were recovering and had no sputum; 1 case was quite incipient. Seven gave the tuberculin test; 4 were negative. In the sputum of 9 of the 11, tubercle bacilli had never been demonstrated; in the remaining 2, acid-

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[28] Gay, F. P., and Force, J. N.: Preliminary Note on a Skin Reaction Indicating Protection Against Typhoid Fever, University of California Publications in Pathology, 1913, ii, 127.

[29] Deilman, O.: Ueber die specifischen Stoffe des Tuberklebacillus und anderen Sauerefester Bacillen, Ztschr. f. Immunitätsforsch., Orig., 1911, x, 421.

fast organisms were demonstrated; Case 33 gave a clear tuberculin reaction in addition to streptotrichin-H; the other—Case 28—an equally distinct streptotrichin-E and no tuberculin reaction, indicating in all probability a double infection with a partly acid-fast and non-acid-fast strain, not with *B. tuberculosis,* as the sputum record would indicate. The granules of *Streptothrix hominis* were found in the sputum of all these cases except two from which no sputum could be obtained. In these two, Cases 30 and 32, all previous examinations of sputum had been negative for *B. tuberculosis.* In Cases 33 and 28, both in advanced stages, it is probable that the secondary invader was *Streptothrix hominis.* This seems to be undoubted evidence of the possibility of the coexistence of the two infections. There is no doubt great variation in the degree of acid-fastness possessed by these organisms, and the exact color limit of a given strain or even of individuals of a given strain will vary correspondingly, so that differentiation by tinctorial methods is impossible. In these skin reactions we are employing the whole product of the culture, the non-acid-fast protein as well as the acid-fast extracts. This should consequently be a more reliable basis for determining specific differences when the reaction is once established in the body than the tinctorial reactions of the micro-organisms which depend wholly on the acid-fast substances. The remaining two cases, 61 and 66, yielding a reaction to streptotrichin-H, were gland infections. The one, Patient 66, a child aged 6, had been operated on for adenitis, presumably tuberculous, two or three days before the test was applied. The patient gave a distinct reaction to streptotrichin-H, but none either to tuberculin or to streptotrichin-E. Six weeks before, large tonsils and adenoids had been removed successfully; no enlarged glands existed at the time of examination. One month later he returned to the hospital with a mass of enlarged glands on the left side. They were subsequently removed, the skin test being made a day or two later. The mass removed was about the size of a hen's egg, hard, discrete, and showed no fluctuation. On section of the tissues no distinct lesions of tuberculosis were evident; there were a few giant-cells but no bacilli or caseous softening, a good deal of round-cell infiltration, and marked density of structure. With the Gram stain rod-segments and fragments of threads of varying lengths were easily found, though none of the granules characteristic of older lesions were present. The case was acute and in the early stage of *invasion,* the tissues being quite generally attacked by the rod segments and threads of the fungus, and it is most probable that not sufficient time had elapsed to make the granules. The close connection between the removal of the enlarged tonsils and the infection of the glands is noteworthy; that the streptothrix was in the tonsillar crypts is most possible, and finding a good portal of entrance in the

TABLE 2.—Reactions in Fifty-Four Abnormal Cases *

No. of Patient	Lung Infection	Gland Infection	Bone Infection	Sputum Finding †	Skin Reactions			Remarks ‡
						Strepto-trichin		
					O. T.	E.	H.	
1	....	Cervical	....	....	+	0	0	
2	Moderate	....	....	T. B. +	+	0	0	
3	Moderate	....	....	T. B. +	+	0	0	
4	Advanced	....	....	T. B. +	0	0	0	
5	Advanced	....	....	T. B. +	0	0	0	Extensive nephritis.
6	Moderate	....	....	T. B. +	+	0	0	Both lungs.
7	Early	....	....	T. B. +	+	0	0	Rapidly advancing.
8	Advanced	....	....	T. B. +	0	0	0	Lung and larynx.
9	*Moderate*	*Scar in neck healed*		*T. B. +*	+	+	*o*	*Glands several year before.*
10	Advanced	....	....	T. B. +	+	0	0	
11	Moderate	....	....	T. B. +	+	0	0	
12	Moderate	....	....	T. B. +	+	0	0	
13	Advanced	....	....	T. B. +	0	0	0	
14	Incipient	....	....	T. B. +	+	0	0	
15	Very slight	Cervical	....	No sputum	+	0	0	
16	Quiescent	....	....	No sputum	+	0	0	No record of T. B.
17	Moderate	....	....	Negative	+	0	0	
18	Slight	....	....	T. B. +	+	0	0	
19	Quiescent	....	....	T. B. 0	+	0	0	No record of T. B.
20	Moderate	....	....	T. B. 0	+	0	0	No record of T. B.
21	Moderate	....	....	T. B. +	+	0	0	
22	Advanced	....	....	T. B. +	+	0	0	
23	Moderate	....	....	T. B. +	+	0	0	
24	*Advanced*	....	....	*T. B. +*	*o*	+	*o*	*Markedly indurate*
25	*Advanced*	....	....	*Negative*	*o*	+	*o*	*Large reaction.*
26	....	....	*Healed knee*	....	*o*	+	*o*	*Childhood infecti eight years befo*
27	*Moderate*	....	....	*Strept. H. +*	*o*	+	*o*	*T. B. never recorde*
28	*Advanced*	....	....	*Strept. H. +*	*o*	+	+	*T. B. recorded sputum.*
29	*Advanced*	....	....	*Strept. H. +*	+	*o*	+	*T. B. never record*
30	*Recovering*	....	....	*No sputum*	*o*	*o*	+	*T. B. never record*
31	*Slowly progressing*	....	....	*Negative*	*o*	+	*o*	*T. B. never record*
32	*Recovering*	....	....	*No sputum*	+	*o*	+	*T. B. never record*
33	*Advanced*	....	....	*T. B. and Strept. H. +*	+	*o*	+	*Abundance of b organisms.*
34	Moderate	....	....	T. B. +	+	0	0	
35	Slight	....	....	No sputum	+	0	0	
36	*Slight*	....	....	*Strept. H. +* *T. B. o*	*o*	*o*	+	*O. T. hypoderm cally; twice asthma.*

TABLE 2.—Continued

No. of Patient	Lung Infection	Gland Infection	Bone Infection	Sputum Finding †	Skin Reactions			Remarks ‡
						Streptotrichin		
					O. T.	E.	H.	
37	*Moderate*	....	....	*Strept. H. +* *T. B. o*	*o*	*o*	+	*T. B. never found.*
38	*Early*	....	....	*Strept. H. +* *T. B. o*	*o*	*o*	+	*Six months; some signs.*
39	*Advanced*	....	....	*Strept. H. +* *T. B. o*	*o*	*o*	+	
51	....	Mesenteric and peritoneal	....	....	?	0	0	Child; advanced.
53	....	Cervical	....	....	+	0	0	Operation one week before.
54	Slight	Cervical	....	T. B. +	+	0	0	Slight cough.
55	....	....	Kyphosis T. B.	....	+	0	0	Since 2 years old; 10 years now.
61	....	*Bronchial*	....	*No sputum*	*o*	*o*	+	*Marked induration in skin; baby.*
62	....	Mesenteric	....	....	+	0	0	
63	....	Cervical	Cervical kyphosis	....	+	0	0	
66	....	*Cervical*	....	....	*o*	*o*	+	*Organisms in glands removed.*
69	....	....	*Dorsal kyphosis slight*	....	*o*	+	*o*	*Obscure myelitis; slight kyphosis.*
71	Moderate	....	....	T. B. +	+	0	0	
72	*Advanced*	....	....	*T. B. o*	+	+	*o*	
74	Healed	....	Dorsal kyphosis	....	+	0	0	All active process over.
79	....	....	....	....	+	0	0	Fistula in ano.
89	*Incipient*	....	....	*Strept. H. +* *T. B. o*	+	*o*	+	*Slight signs.*
91	....	Healed cervical infection	....	....	+	0	0	Eleven years before.
96	....	Healed cervical infection	....	....	+	0	0	Glands removed three years ago.

[*] In this table + indicates a reaction. 0 indicates no reaction. T. B. = *B. tuberculosis,* and O. T. = Koch's tuberculin. † From personal examination. ‡ From sputum reports from other records.

wound left by the first operation it rapidly invaded the lymph-glands. It was very regrettable that cultures were not made from the gland tissue before hardening.

Case 61 was that of a child of 13 months, admitted to the hospital with acute intestinal decomposition and a condition of malnutrition of seven months' standing. There was no recognizable lesion, but a roentgenogram showed very large bronchial glands. A distinct positive reaction to streptotrichin-H was given by this child; tuberculin and streptotrichin-E were both negative. Although unfortunately not possible of corroboration, this is presumptive evidence of a streptothrix infection. Case 89 was incipient; a slight cough and expectoration had lasted for six months, a slight loss in weight and a general below-par condition indicated some trouble. Tubercle bacilli had been looked for without result. The skin test gave a slight reaction to tuberculin and also a slight one to streptotrichin-H. The latter was not large, but quite recognizable. The patient was a dentist by profession and could in consequence have been subjected to repeated opportunities of infection.

STREPTOTRICHIN-E REACTIONS

Eight cases, 9, 24, 25, 26, 28, 31, 64 and 72, yielded a reaction to streptotrichin-E. Six were lung involvements, two bone diseases. Of the lung cases three were clear-cut: no tubercle bacilli in the sputum, no tuberculin reaction, and a definite streptotrichin-E reaction, without doubt infections with a less acid-fast organism than *B. tuberculosis*. Of the remaining three cases, two gave double reactions to tuberculin and streptotrichin-E. Of these one had tubercle bacilli in the sputum and scars of an old cervical gland trouble, while the other had no tubercle bacilli and was probably to be put in the same class as the first three. The sixth case gave a double reaction to the two streptotrichins and a negative reaction to tuberculin, with a record of tubercle bacilli and *Streptothrix hominis* in the sputum. Again, a more acid-fast streptothrix rather than true *B. tuberculosis* was probably the infecting agent.

Few data are available to show how long these reactions will last after recovery from infection. Case 91, streptotrichosis of neck eleven years before, with a negative reaction, is the only record. It may be relatively transient. The double reactions between either end and the middle member of our series might be looked on as group reactions (Feistmantel[30]) if it occurred invariably or if no corroborative evidence were possible as to the nature of the infecting parasite. The tuberculin reaction in the absence of tubercle bacilli is of no value as evidence in favor of such infection, in view of its general occurrence in many apparently healthy cases. Of the two cases reacting to both tuberculin and streptotrichin-E, Case 72 had no record of *B. tuberculosis* in the sputum, while Case 9 had bacilli in the sputum with a recent lung and old gland involvement. The remaining case, No. 28, with both streptotrichin reactions, exhibited both acid-fast organisms and streptothrix granules in the sputum, with no tuberculin reaction. This was then probably a case of double infection with both organisms, a partly acid-fast and a non-acid-fast type. The latter seems especially likely to have been the secondary invader. It is not possible at present to clear away all doubts as to diagnosis in these cases of infection with the partly acid-fast forms, but it is of some assistance to be able to differentiate the end members, and evidence from these observations seems to justify this. Case 86 was a case of blastomycosis, and it is interesting to note that it was negative to streptotrichin and sporotrichin but positive to blastomycotin.

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[30] Feistmantel: Die Tuberkulinreaktion Ein Beitrag zur Feststellung ihre Wesens als Gattungsreaktion, Centralbl. f. Bakteriol., 1904, xxxvi, 282, 406. Experimenting with guinea-pigs infected with *B. tuberculosis* and *Streptothrix nocardii,* Feistmantel found an equally severe reaction on injecting subcutaneously either O. T. or an analogous preparation from the *Streptothrix*. He concludes that the reaction is non-specific. It is to be remembered that his tests were subcutaneous and the doses of relatively large size.

THERAPEUTICS

Three lines of treatment, singly or combined, have been applied to streptotrichosis: surgical removal of the infected part, vigorous internal use of some form of iodin or copper sulphate, and specific vaccine therapy. The surgeon unaided cannot hope to cure unless he makes an early diagnosis, since the spread of the organisms along lymphatics and blood-vessels may have occurred; only in cases sharply localized like gland infections or appendiceal cases can the whole area involved be removed. The markedly favorable influence of iodin on some cases of all fungoid infections, blastomycosis, streptotrichosis and sporotrichosis is well known, and especially is this true in the earlier, more curable stages. Some of the more advanced ones also respond significantly to it. Iodin in the form of potassium or sodium iodid, changing even to hydriodic acid when intolerance is exhibited, are the usual preparations. The amount taken should be increased daily until marked symptoms of iodism appear, and then either changed in form or reduced in amount and again increased; the presence of a maximum amount of iodin in the body is apparently unfavorable to the fungus. At first there is usually a noticeably larger amount of pus and sputum from the diseased area and a marked increase in the number of organisms present. This is followed by a reduction of both discharge and organisms, and, if the case is a curable one, of a gradual healing of the tissues. Copper sulphate in ⅓-grain doses three times daily has also been employed in the treatment of these cases. Like the iodids, it is liable to disturb the stomach, and it is doubtful whether it has a specific effect on the fungus; but it is a useful alternate to the iodids.

Specific treatment in the form of vaccines yields variable results; but the number of cases observed and treated is too few to make a basis for safe generalization. Petruschky[31] in 1898 found a case of streptotrichosis in a schoolgirl aged 12, suspected of being tuberculous, whose mother and older sister had died of consumption, but who had no "demonstrable bacilli." A streptotrichin similar to Koch's tuberculin was prepared and used, and recovery followed. Cohn made a normal salt suspension of an agar-slant (1 c.c. to a culture), diluting 100 times. Of this he gave 0.3 c.c. subcutaneously with no local reaction, repeating an increasing dose on each third day, increasing 0.3 c.c. each time. The case was an advanced prostatic infection, one with many secondary disturbances and too poor a general condition to allow of any surgery. Under the treatment, the prostate was reduced to a small size, but the patient died from general causes. In 1911 Harbitz and Grondahl[32] attempted treatment with vaccines, with one recovery and one death. Their attempts to immunize animals were unsuccessful, all serum reactions being negative. It is probable that their preparations made from the cultures were defective. Normal salt suspension may not contain enough bacterial protein to call out immune bodies in experimental animals. An endotoxin extraction according to Besredka's and Gay's method[25] produces a highly successful preparation for this purpose. Williams[18] made a vaccine from the acid-fast type of *Streptothrix leproides,* which was at the time of writing (1911) proving quite successful in cases which had soft, "juicy" lesions with a good blood-supply. These lesions regressed noticeably, and fresh exacerbations and extensions of the disease were apparently checked. Ultimate cure Williams does not claim; time alone can demonstrate that fact. He thinks that the vaccine has better results than the nastin treatment of Deycke; having used both he is in a position to judge.

Kinnicutt and Mixter[33] used a vaccine in eight cases. It was made from autogenous cultures grown in bouillon, and when of sufficient size the masses were fished out and transferred to a sterile test-tube, broken up as much as possible with a glass rod, then heated to 60 C. for one hour, transferred to 50 c.c. of sterile salt solution and 0.1 c.c. used for first dose, repeated in three or four days with an increase of 0.05 or 0.1 c.c. each time. Eight cases were treated by Kinnicutt and

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[31] Petruschky: Demonstration von Preparaten und Kulturen von einem zweiten intra-vitam diagnostiziesten Falle von Streptotrichosis hominis, Deutsch. med. Wchnschr., 1898, xxiv, 78 v (Vereins-Beilage).

[32] Harbitz, F., and Grondahl, N. B.: Actinomycosis in Norway: Studies in Etiology, Modes of Infection, Treatment, Am. Jour. Med. Sc., 1911, cxlii, 386.

[33] Kinnicutt and Mixter: Actinomycosis Treated with Vaccines, Boston Med. and Surg. Jour., 1912, p. 90.

Mixter—two abdominal, two pulmonary and four cervicofacial. The pulmonary cases were advanced, and no gain was shown under treatment, but for the first few days a great increase in the granules in the sputum occurred. Of the two abdominal infections, one patient was cured and one died. Three of the jaw and neck patients recovered, and one had almost recovered at the time of writing. Kinnicutt and Mixter conclude that vaccines are useful in *local* infections, but are not of marked effect in deep-seated or advanced cases.

The widely differing preparations used, the relatively few cases recorded, and especially the lack of diagnosis of early infections, leaves but a slight basis for estimating either the curability of the disease or the best methods of treatment. There is no doubt, however, that early recognition and prompt treatment, either by iodin or vaccine, would help more of the patients to recovery than are at present cured.

CONCLUSIONS

1. Streptotrichosis is more common than generally supposed and frequently not differentiated from other infections; especially is it confused with tuberculosis. Clinical signs are not characteristic enough to determine the diagnosis accurately.

2. Discharges—sputum, pus, etc.—should be carefully examined fresh in as large quantities as possible, and smears stained with carbolfuchsin and Gram stains. The amount of decolorization in the carbolfuchsin preparations should be varied so as to differentiate the less acid-fast types.

3. Streptotrichins (glycerinated bouillon cultures, concentrated), made from the non-acid-fast mycelial and partly acid-fast bacillary organisms, give definite skin reactions on persons with frank infections, but do not show on controls (persons presumably non-infected) including cases of recognized tuberculosis. This either shows the infection to be less general than that due to the tubercle bacillus or else the reaction to be a less long-lasting one.

4. Streptotrichosis and tuberculosis may exist in the same person at the same time, as evidenced by the appearance of both organisms in the sputum and both skin reactions.

5. Gland and bone infections with streptotriches may be demonstrated by skin reactions as well as lung infections. This still further broadens the field for confusion between the tuberculoses and streptotrichoses.

I wish to express my thanks to the physicians in charge of the Alameda County Society for the Study and Prevention of Tuberculosis of the University of California Hospital, and of the Alameda County Hospital for their courtesy in allowing me to use clinical material in these institutions.

A PRELIMINARY REPORT ON THE PRODUCTION, ACTION AND THERAPEUTIC EFFECTS OF LEUKOCYTIC EXTRACTS *

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Subsequent to the publication of an article by Hans Zinsser, George W. McCoy and C. W. Chapin[1] on The Protective Influence of Leukocytic Substances on Experimental Plague, one of us conceived the idea that if leukocytic substances could be obtained in sufficient quantities to be not only therapeutically practical, but commercially possible, a decided step forward would be made in the biological treatment of infectious diseases.

Prior to the publication of the above mentioned article, Hiss and Zinsser[2] demonstrated in an experimental way that leukocytic extracts could be prepared by injecting into the pleural cavities of rabbits 10 c.c. of a meat extract broth containing 3 per cent. starch and 5 per cent. aleuronat. At the end of twenty-four hours a copious, and very cellular exudate will have accumulated in the pleural cavities. This is obtained by killing the rabbits, and removing the exudate which is immediately centrifuged. The leukocytic sediment is diluted with twenty volumes of sterile distilled water to one volume of sediment, and autodigestion is permitted to take place in the incubator, following which the extract is stored in a refrigerator until used.

In experimenting with animals, Hiss and Zinsser observed that pneumococcus, staphylococcus, streptococcus, meningococcus, typhoid, dysentery, and cholera infections in rabbits and guinea-pigs, were profoundly modified when injections of leukocytic extracts prepared as above described were administered intraperitoneally or subcutaneously. during the course of the infection. In many cases, animals were saved by these substances from infections which proved rapidly fatal in untreated control animals, even when the protective injections were made as late as twenty-four hours after intravenous infections.

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* From the Research Department of the Western Laboratories, Oakland, Cal.

* Read before the Medical Research Society of the East Bay Counties, Oakland, Cal., April 4, 1914.

* Submitted for publication, April 30, 1914.

[1] Zinsser, Hans, McCoy, Geo. W., and Chapin, C. W.: The Protective Influence of Leukocytic Substances on Experimental Plague, Jour. Med. Research, 1911, xxiv, No. 3.

[2] Hiss, P. H., and Zinsser, Hans: Jour. Med. Research, 1908, xiv, No. 3; 1909, xv, No. 3.

In applying this method of treatment by subcutaneous injections to infections in man, Hiss and Zinsser observed distinctly beneficial results in cases of epidemic cerebrospinal meningitis, lobar pneumonia, staphylococcus infections, and erysipelas.

Hiss and Zinsser's idea was that injecting into infected subjects the substances composing the cells usually found in exudates in a diffusible form, such substances would, following absorption, relieve or stimulate the fatigued leukocytes.

Later, Zinsser, assisted by McCoy and Chapin, conducted a series of experiments on white rats with a view to ascertaining whether substances obtained from normal leukocytes would modify in any way the course of experimental infection with the bacillus pestis. As the results of their experiments these workers found that the use of emulsions of normal leukocytic substances obtained from rabbits exerted a distinctly protective influence, even when such substances were administered twenty-four hours after the inoculation of rabbits with virulent plague organisms.

While the results obtained by the above mentioned investigators were intensely interesting from a practical point of view their method of obtaining leukocytic substances must be considred as of little value, since the injection of a protein substance into the pleural cavities of rabbits yields such a small amount of leukocytic substance as to rob it of any value therapeutically.

Furthermore, the leukocytes so obtained are, strictly speaking, inflammatory leukocytes, which have probably given up much of their potential qualities, or in other words, have become fatigued or exhausted with the task of caring for the foreign protein, the injection of which caused them to migrate into the pleural cavity.

For the purpose of throwing some light on the nature of bactericidal substances in leukocytic extracts, Wilfred H. Manwaring[3] conducted a series of experiments. Manwaring started out with the assumption that there exists powerful bacteriolytic substances within the cytoplasm of certain body cells, and that this fact is amply demonstrated in the automatic sterilization of old abscess cavities and the sterilizing of the pneumonic lung, which is assumed to be due to the liberation of endolysins, the result of cellular disintegration. His work was done with a view of extending the present knowledge of these lytic substances, and to determine the approximate chemical nature of the bacteriolytic substances that might be extracted from leukocytes.

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[3] Manwaring, Wilfred H.: The Nature of the Bactericidal Substances in Leukocytic Extract, Jour. Exper. Med., 1912, xvi, No. 3.

In obtaining leukocytes for these experiments, Manwaring at first followed the practice of injecting a 5 per cent. suspension of aleuronat into the pleural cavities of rabbits, but he found that he was decidedly handicapped in his study of the nature of the extracts so obtained on account of the small amount of substance available. By injecting aleuronat into the pleural cavity of a rabbit, one seldom secured more than a single cubic centimeter of leukocytes. In order to overcome this obstacle, he selected the horse for the continuation of his work. His treatment consisted in injecting into the pleural cavity of a horse, from 300 to 500 c.c. of a 5 per cent. aleuronat in 2 per cent. starch-paste solution, the starch being added to aid in holding the aleuronat in suspension. Following such an injection there is usually found from 1 to 3 liters of pleural exudate daily for the first week; the daily yield then decreases, usually ceasing entirely about the fifteenth day. The exudate contains about 5 per cent. of leukocytes.

Manwaring, while not being able to arrive at a final conclusion as to the nature of the bactericidal substances in leukocytic extracts, did reach the logical conclusion, as the results of his investigations, that the bactericidal substance present is probably an enzyme.

We could, did circumstances permit, go on and recount the results obtained by other investigators, showing that leukocytes do contain intracellular substances which possess proteolytic properties, but from a study of the question from a therapeutic standpoint, the results obtained by later investigators, and our own personal work, indicate that the direct bactericidal action brought about by the parenteral injection of leukocytic substances does not influence to a marked degree the progress of an infectious disease. While able to demonstrate *in vitro* bactericidal properties in leukocytic extracts produced by the method devised by us, it was long ago concluded that such bactericidal powers were not sufficient to account for the profound changes actually produced in animals suffering from infectious diseases while undergoing a course of treatment by the parenteral injection of leukocytic substances. For the reason of these profound changes we must look elsewhere than to the direct lytic action on the invading bacteria.

It is a well-established fact that the parenteral injection of protein substances increases the leukocytic count, and even the injection of foreign vegetable proteins such as nuclein produce, in many infections, marked beneficial effects. It would seem, however, in carrying out leukocytotherapy, that in order to produce a hyperleukocytosis without unnecessary strain on the organism, it is eminently more logical for the organism to care for parenteral injections of substances similar to its own elements than to any elements which are of an alien character.

It is also a well established fact that in the treatment of diseases, any method which will stimulate the leukocyte-producing organs to a more active function, thereby producing a leukocytosis, is and must of necessity be of positive benefit. The great problem before us then in this connection is to ascertain the simplest and most innocent means of accomplishing this end.

About the beginning of the year 1911 a series of experiments were instituted with a view of obtaining leukocytic substances in quantities that would render them not only therapeutically practical, but also commercially possible. The general plan adopted was to obtain leukocytes directly from the blood of normal animals, avoiding as far as possible any changes in their character during the process. It is unnecessary to go into detail as to the various methods employed to bring this about. Suffice it to say that after much experimental work, the following general technic was adopted:

TECHNIC

The blood is obtained under aseptic precautions from the jugular vein of the horse, dog, or other domestic animal; is allowed to flow into sterile flasks containing a sufficient quantity of 1 per cent. sodium citrate solution to prevent coagulation. When the blood arrives at the laboratory, sodium citrate solution is added in varying amounts, depending on the species of animal from which the blood was obtained; to this is added five-tenths of 1 per cent. acetic acid, the necessary amount of which also depends on the character of blood being treated. The mixture is then centrifuged in a high powered centrifuge and the supernatant fluid is discarded. The sediment, which is very rich in leukocytes, is washed several times with physiological salt solution, ground with quartz sand and neutralized. To the resulting sediment distilled water is added in the proportion of about four volumes of water to one of sediment. This mixture is exposed to a temperature of 58 C. for one hour, after which it is placed in the incubator until digestion of the leukocytes is complete. It is finally centrifuged, the supernatant fluid decanted and sufficient trikresol added for preservative purposes. Each lot prepared as described is carefully examined bacteriologically and its ability to produce certain definite blood changes is determined by animal inoculation.

While a great many experiments have been carried out, not only on different animals, but also on human beings, for the purpose of shedding some light on the action brought about by the parenteral injection of leukocytic extracts, it is obviously unnecessary and impracticable to submit the great mass of data so obtained at this time. We wish, however, to present the results of a few experiments which will suffice to demonstrate some of the blood changes that follow injections of the extracts.

EXPERIMENTS

Experiment 1.—For the purpose of ascertaining the different effects, if any, following parenteral injections of leukocytic substances obtained from various animals into a series of animals of the same species, with a view of determining whether the extracts of a different species will give rise to the same changes as leukocytic extracts from the same species, the following experiment was instituted:

Four guinea-pigs of equal weight were injected as follows: Pig 1 received 1 c.c. of horse leukocytic extract; Pig 2, 1 c.c. of dog leukocytic extract; Pig 3, 1 c.c. of guinea-pig leukocytic extract, and Pig 4, 2.5 c.c. nuclein (P. D.).

Chart 1 shows their total leukocytic curves and the total polymorphonuclear counts.

It will be observed that the injection of leukocytic extracts obtained from three different sources as well as the nuclein produced a marked leukocytosis. The leukocytic substances, with the exception of the guinea-pig extract, produced a greater reaction than the nuclein.

The dog extract produced the most marked reaction, the total leukocytic increase amounting to about 300 per cent. The differential counts showed that the dog leukocytic extract caused the most profound changes, the neutrophil increase amounting to 350 per cent., and the eosinophil count increasing from 3 to 14 per cent. The large mononuclears showed, in many cases, bundles of chromatin in their protoplasm. The nuclei of these cells were crowded to one side with a loose net-work of chromatin in the protoplasm on the opposite side. These cells appeared about one and one-half hours after the injection; gradually increasing in number for about six hours, when about one-third of the cells showed this change. In the case of the guinea-pig injected with guinea-pig leukocytic extracts, the polymorphonuclear

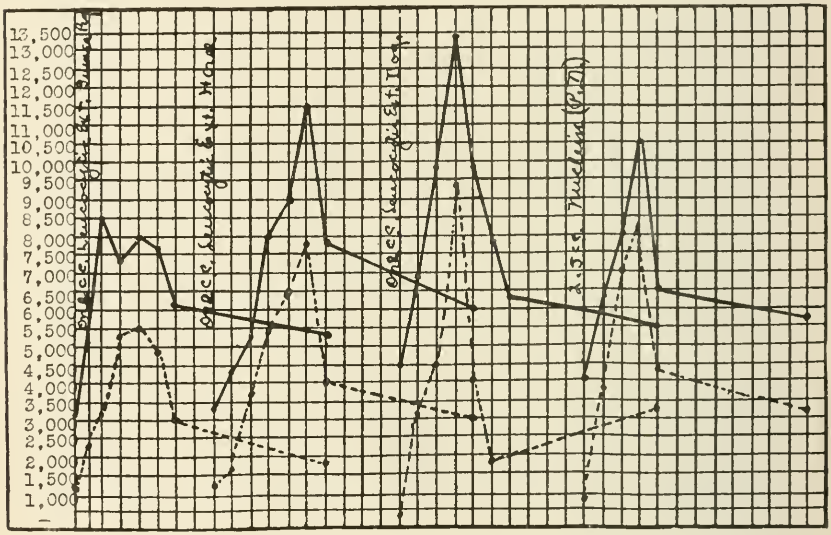

Chart 1.—Guinea-pig series. Each square represents a one and one-half hour interval. In this and following charts the solid line indicates total white count; line of short dashes, total polynuclear count; dotted line, total neutrophil count; dash and dot line (—.—.—.—.), total eosinophil count.

leukocytes showed practically no granules and no red staining before the injection was made. A few red granules were noticed at the second count and at the third count, with but few exceptions, all the neutrophils showed distinct red granules in their protoplasm. These granules decreased in size and number until they had practically disappeared at the twenty-fourth hour after injection.

In the guinea-pig injected with horse leukocytic extract, the polymorphonuclear leukocytes showed no red granules before injection, but at the second count the red granules were at their height, disappearing at the third count, a few reappearing on the following day.

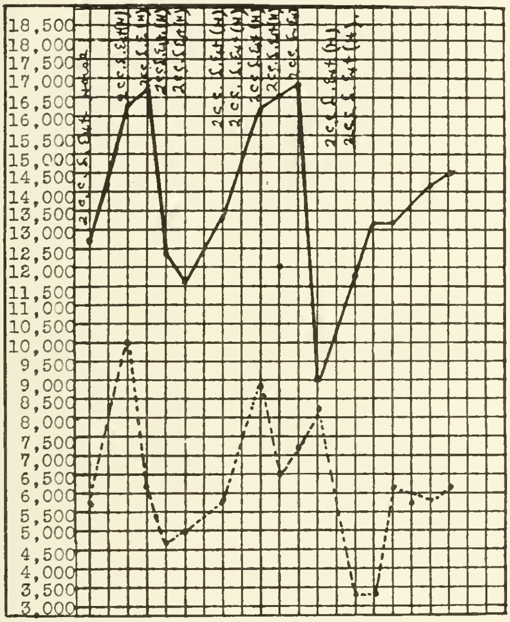

Chart 2.—Rabbit 1. Each square represents twenty-four-hour interval.

No counts could be made from the nuclein-treated pig until four and one-half hours after the injection, due to the fact that the blood clotted so rapidly. At this time a few red granules were observed in a few of the cells, but this phenomenon was not nearly so marked as in the animals injected with the leukocytic extracts.

Experiment 2.—Chart 2 shows the daily blood picture of Rabbit 1, which received twelve subcutaneous injections of 2 c.c. horse leukocytic extract at twenty-four and forty-eight-hour intervals. Blood-counts were taken daily, commencing at the time of the first injection, and continued for fifteen days. Smears made at the time of the first injection showed the protoplasm of the neutrophils, with a few exceptions, to be free from granules, the granules in these exceptions being very fine and taking a distinct red stain. At no time after the injections of the extract were the granules as fine, nor as few in number as before. Practically all the polymorphonuclear cells contained large, distinct, red granules; these granules had not decreased in number seventy-two hours after the last injection, at which time observations ceased.

It will be observed that marked total leukocytic curves occurred throughout the experiment. Differential counts showed that the increase in the eosinophil count and decrease of the neutrophil count, and the increase of the neutrophil count and the decrease of the eosinophils kept pace with the rise and fall of the total leukocytic count. When the leukocytes took an upward curve the eosinophils also increased, reaching at one time a height of 45 per cent.

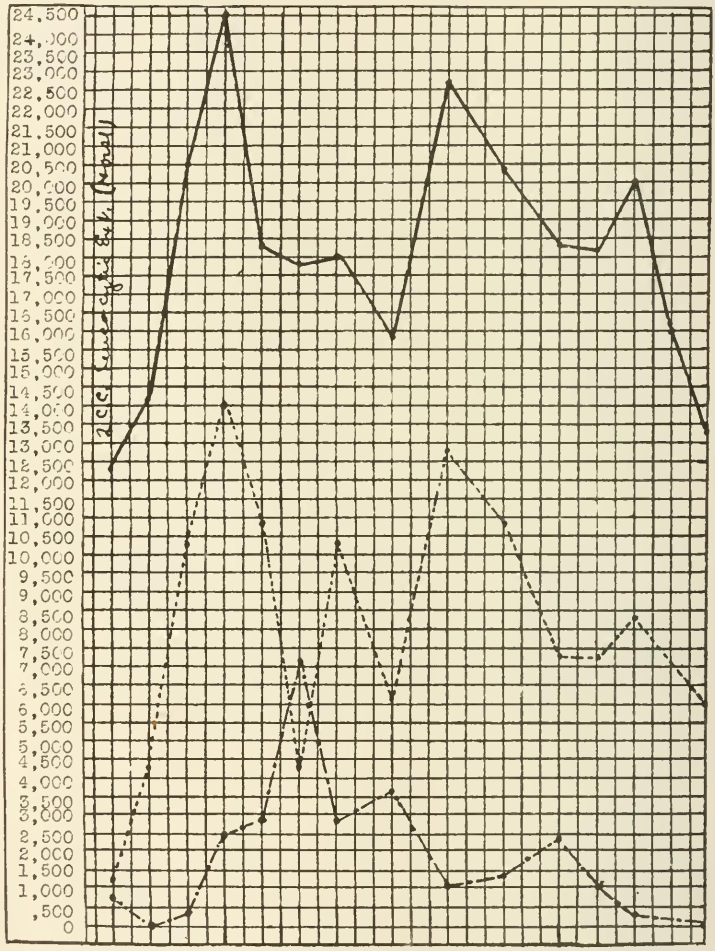

Chart 3.—Rabbit 2. Each square represents a one-hour-interval.

Experiment 3.—Rabbit 2 was given a single injection of 2 c.c. horse leukocytic extract. Blood-counts, as shown in Chart 3, were taken at two-hour intervals for thirty-six hours. The neutrophils showed much the same normal picture as in Rabbit 1. The red granules began to appear four hours after injection, and increased to a maximum in size and number between the fourth and sixth hours. These granules did not disappear during the time of the experiment, but decreased in size and number from the tenth hour on. The total count showed a drop during the first two hours, and then took an upward curve reaching the maximum height of 100 per cent. above the normal about the sixth hour. The differential counts showed an increase and decrease of the eosinophils and neutrophils coincident with the curves formed by the total leukocytic counts. The total neutrophil count at its maximum reached a point of 700 per cent. above the normal and the eosinophil total count 1,100 per cent. above the normal.

Experiment 4.—With a view of demonstrating the effects of leukocytic extracts on a human being the following experiment was carried out:

Chart 4 shows the blood pictures of a man subsequent to a subcutaneous injection of 2 c.c. of dog leukocytic extract.

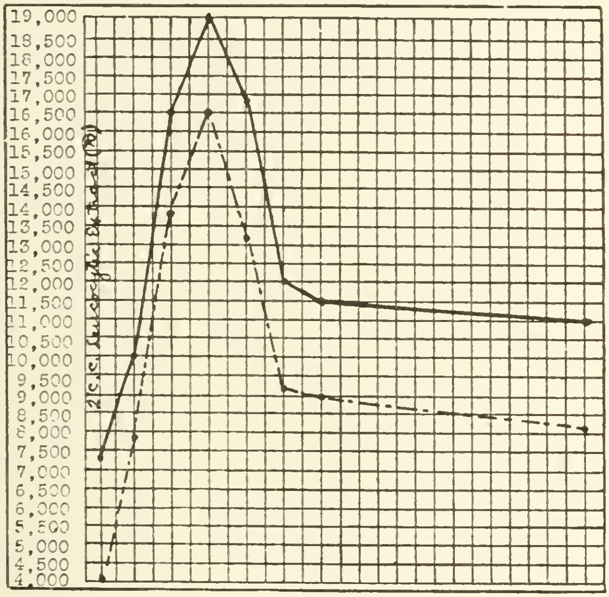

Chart 4.—Normal man. Each square represents a one-hour-interval.

A white and differential count was made just prior to the injection and subsequent counts were made at two-hour intervals for a period of twelve hours. A final count was made twenty-six hours after the injection.

The curve showed a continual increase beginning two hours after the leukocytic extract was administered, reaching its maximum at about the sixth hour. At this point the total leukocytic count was slightly over 150 per cent. above the normal and the polymorphonuclears had increased to about 500 per cent. From the sixth to the twelfth hours there was a gradual decrease both of the total leukocytic and polymorphonuclear counts. Twenty-six hours after the injection, the count had not changed from that found at the twelfth hour. At this time the total leukocytic count was about 50 per cent. above the normal, with a polymorphonuclear count a little over 100 per cent. above the normal.

The therapeutic application of leukocytic extracts to humans and animals has been followed out during the past three years. Various infections have been treated, in some types, more especially acute infections, with most gratifying results, while in others, especially old chronic infections, the beneficial results were not so marked. Some of the diseases of human beings to which the treatment has been applied, and which have yielded the most pleasing results, are pneumonia, puerperal sepsis, erysipelas, empyema, bacteremia, acute pyemia, gonococcus infection, rheumatism, etc.

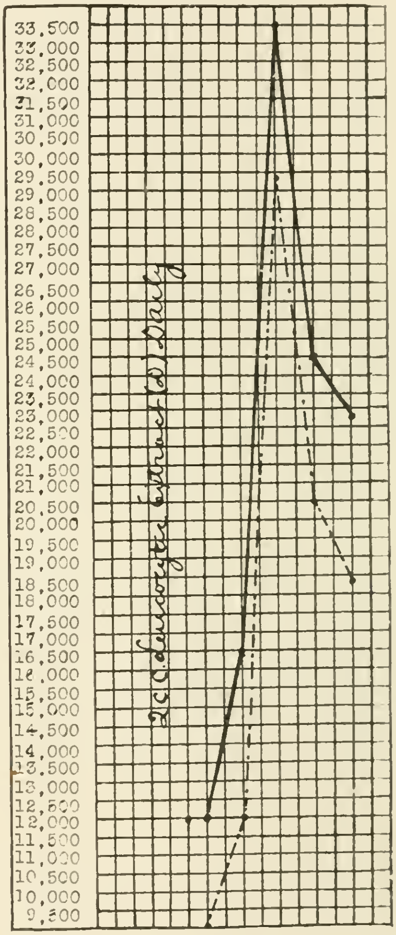

Chart 5.—Lobar pneumonia (left lower lobe). Each square represents a twenty-four-hour interval.

The following are a few of the records selected from a number of human cases treated with leukocytic extracts:

CASE REPORTS

Case 1 (Chart 5. Lobar Pneumonia).—Miss M., a Portugese school-girl aged 16, was first seen at 2 p. m., March 10, at which time she gave a history of a severe chill twenty-four hours previously. This was followed by fever, headache, nausea, vomiting, and pain in the left chest. Examination showed a temperature of 103.6 F., pulse 112, respiration 36, white blood-count 12,000, polymorphonuclear cells 70 per cent. with a dull area over the lower lobe of the left lung. Two cubic centimeters of leukocytic extract was given once daily for eight days. Following the first dose of extract the white blood-count rose to 33,400 with 88 per cent. polymorphonuclear cells, and the total count remained above 23,000 during the remainder of her illness. After the third dose the temperature came down by lysis, reaching normal on the eleventh day of the disease.

Case 2 (Chart 6. Cellulitis).—The patient, a young man, when first seen complained of a swelling, redness, and pain in the right arm and adjacent chest wall. The constitutional symptoms of nausea, vomiting and headache were severe, the temperature was 103 F. and there was no tendency to localization or pus formation. At the time of admission to the hospital the white blood-count was 25,000; unfortunately no differential was made.

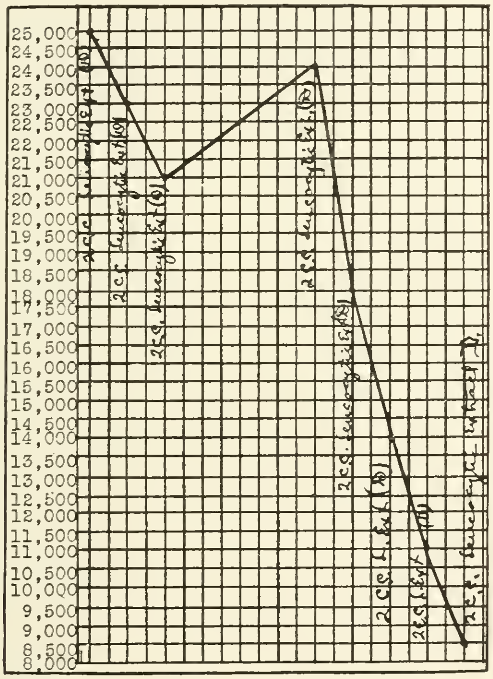

Chart 6.—Cellulitis. Each square represents a twenty-four-hour interval.

The treatment at this time consisted in the subcutaneous injection of 2 c.c. of leukocytic extract once daily, with the exception of the second dose, which followed the first after a period of twelve hours. Both the local and general symptoms were very much improved after the second dose and on the third day the temperature was normal, the patient felt well except for a slight tenderness in the affected part, the skin was normal in color, but there still remained some swelling. On account of the improved condition, it was thought best to discontinue the use of the extract at this time. That the treatment should have been continued longer was evidenced by the fact that on the seventh day, which was the fourth day without treatment, all the local and general symptoms returned, following a sudden and severe chill. The temperature again reached 103 F. and the total leukocyte count 24,000. Two cubic centimeters leukocytic extract were again given once daily for the next six days. The general symptoms as before, quickly improved, and following the second dose the infection became localized and an abscess formed which was drained surgically. Healing was rapid and on the twelfth day after admission the patient left the hospital well. The chart accompanying this record is most interesting. It will be noticed that the high blood-count on admission decreased after the first doses of the extract, and increased when the extract was discontinued, to again decrease as soon as the treatment with the extract was resumed. As no blood-counts were made on the fourth, fifth and sixth days, the lowest blood-count reached following the first doses of extract is not known.

Case 3 (Puerperal Sepsis).—On the third day following confinement Mrs. G. developed a temperature of 104 F. with nausea, vomiting and intense headache. On the following day she had several slight chills, with temperature continuing high, and accompanied by some delirium and later stupor. The patient was then removed to the hospital and 2 c.c. leukocytic extract given daily. The following day she was brighter, said she felt better, temperature dropped to 102 F. There was no further delirium, improvement continued, and the temperature reached normal four days after the administration of the extract. A blood culture made at the time this patient entered the hospital showed the presence of the *Staphylococcus aureus.*

Case 4 (Empyema).—Mr. B. injured by a fall from a telephone pole Oct. 17, 1913, was removed to the hospital, Oct. 22, when an abdominal operation was performed for the relief of a general peritonitis. Later an empyema developed which was operated on November 20. The temperature, which had ranged from 102 to 104 F. up to this time, fell to normal following the removal of the pus from the pleural cavity, but the pyothorax quickly returned and the temperature again rose, necessitating a second aspiration. A glycerin-formaldehyd solution was introduced after each aspiration. Up to December 13, when the daily subcutaneous injections of leukocytic extract were commenced, the temperature had ranged between 101 and 102 F., except for brief intervals following drainage. From this time on, however, the temperature fell slowly, reaching the normal on December 28, when the patient reported feeling well and on December 30 left the hospital with no signs of empyema.

Case 5 (Facial Erysipelas).—Mr. S., aged 60, when first seen presented an erysipelas of three days' duration involving the entire face, neck and both ears. His temperature at this time was 105 F. The treatment consisted of daily subcutaneous injections of 2 c.c. leukocytic extract. After the second dose the inflammation ceased to spread, the affected area became lighter in color, and the temperature fell to normal. On the twelfth day after the onset the patient returned to his work, while in previous attacks he had been confined to his bed for from eighteen to twenty-one days.

Some interesting data concerning "Clinical Studies on the Curative Action of Leukocytic Extracts in Infective Processes" by Hiss and Dwyer,[4] are worthy of note in this connection. In this article the results obtained by the use of leukocytic extracts on a number of cases of erysipelas are tabulated.

Total number of cases treated, 148.
Recovered, 143; mortality = 3.38 per cent.
General average duration of treatment, 3.18 days.
Number of recoveries of patients under 1 year old, 8 out of 12.
Percentage of recoveries of patients under 1 year old, 66.6 per cent.
Mortality among patients over one year, 0.73 per cent.

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[4] Hiss and Dwyer: Clinical Studies on the Curative Action of Leukocytic Extracts in Infective Processes, New York Med. Rec., 1913, lxxxiv, No. 11.

These authors also refer to the results obtained by Dr. Adrian Lambert who treated fifty cases of erysipelas at the Bellevue Hospital and obtained 50 per cent. recoveries of babies under one year. Dr. Lambert concluded from his experience that leukocytic extracts will abort infections which are treated within twenty-four hours. Older cases are ameliorated and may abruptly terminate. Toxic symptoms, delirium, headache, nausea, vomiting and pain are relieved. Rash does not disappear immediately, but is apt to localize.

It is fully realized that these therapeutic agents are at present undergoing the "acid test" and it is difficult to prevail on the therapeutist to give them a trial, unless as a last resort. Hence the list of infections for which we can conscientiously recommend the treatment is more limited than it should be. It is believed, however, from our experience in the treatment of animal diseases, that leukocytic extracts have a wide range of therapeutic application and that their use is warranted in all acute infections at least.

In animals the treatment has been applied to a great variety of local and general infections; such diseases of the horse as pneumonia, purpura, influenza, laminitis, strangles, and many local infections have yielded more rapidly to the use of the extracts than to any other treatment heretofore applied. In such diseases of the dog and cat as pneumonia, so-called distemper, and the very common bowel infections in these small pet animals, the results have been most gratifying.

In some of the above mentioned diseases in animals the agent seems to act as a specific, if we may be permitted to use the term. Apparently hopeless infections, even those in which the mortality is ordinarily very high, make good recoveries following daily injections of the extracts.

In such cases as purpura in the horse and distemper in the dog, where the treatment is commenced before the animals have lost their reactive powers, about the third day following the daily injections of the extracts rapid improvement in the clinical manifestations are observed, and this improvement continues until recovery is complete. provided the treatment is kept up until immunity has been well established.

It has been invariably observed that the first and second injections of leukocytic extracts have the tendency slightly to aggravate the toxic symptoms, even to the extent of producing slight uneasiness on the part of the affected animal, but these conditions are soon followed by improvement. These observations would seem to demonstrate that the initial effect of the extract is to increase intoxication by the liberation of toxic elements, which, providing the individual is capable of reacting, stimulate the machinery of immunization and the consequent production of immune elements in sufficient quantities to control or overcome the infection.

We fully realize that there are a great many problems incidental to the production, nature, and effects of leukocytic substances that require solution, but we believe that what has thus far been accomplished may prove to be of some assistance in future study and investigation. We also believe that the facts already obtained warrant the following conclusions:

CONCLUSIONS

1. Leukocytic substances possessing positive therapeutic value may be procured from leukocytes obtained directly from the blood of normal animals.

2. Leukocytic substances may be obtained directly from the blood of normal animals in quantities which render them not only therapeutically practical but commercially possible, conditions that can hardly be claimed for leukocytic extracts prepared from inflammatory leukocytes.

3. The parenteral injection of leukocytic substances causes profound changes in the blood, producing a marked leukocytosis and marked changes in the individual leukocytes. As to the significance of these phenomena no opinion can be offered at this time.

4. Leukocytic extracts have not sufficient direct bactericidal powers to account for the profound changes produced following their parenteral introduction into an animal suffering from an infectious disease, and the beneficial results obtained are probably due to the fact that the extracts furnish elements which stimulate the fatigued or exhausted leukocytes, enabling them to carry on their function of phagocytosis and produce the changes incidental thereto.

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A COMPARISON OF BANG'S MICROMETHOD FOR DETERMINING BLOOD SUGAR WITH BERTRAND'S METHOD *

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It is recognized that the constituents of the blood are of as great a clinical significance as the constituents of the products of excretion, but heretofore blood analysis has not been undertaken as a matter of routine in the clinic, on account of the technical difficulties in the methods employed or because of the large amounts of blood required. Folin and Denis,[1] however, have recently described relatively simple and accurate ways for estimating the non-protein nitrogen, urea, ammonia, and uric acid of the blood, and Marshall[2] has published a method for determining blood urea. These various tests are proving of great value, because clinicians are now able to estimate the nitrogen-containing elements of blood as part of the ordinary study of different types of disease.

Since Claude Bernard[3] recognized that glucose could be found in the circulating blood, a large literature on blood-sugar has accumulated. Two methods for blood-sugar determinations have been generally used. After the proteins from the blood have been removed, the sugar in the filtrate has been quantitated by polarization, or by one of the metallic reduction tests.

The blood proteins have been taken out by numerous reagents. Bernard and Röhmann[4] chose sodium sulphate, Abeles[5] selected an alcoholic solution of zinc acetate or chlorid, precipitating the zinc by sodium carbonate and washing the sugar into the filtrate. According to Schenck,[6] the proteins could be precipitated with mercuric chlorid, which was removed by hydrogen sulphid. MacLeod[7] concluded in later experiments, however, that certain substances in addition to the proteins were brought down by the mercury and that therefore the results so obtained were not absolutely accurate. He advised the use of

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* Submitted for publication, March 18, 1914.

[1] Folin and Denis: Jour. Biol. Chem., 1912, xi, 527.

[2] Marshall: Jour. Biol. Chem., 1913, xv, 487.

[3] Bernard: Leçons de physiologie, 1855, Baillière, Paris.

[4] Röhmann: Centralbl. f. Physiol., 1890, iv, 12.

[5] Abeles: Ztschr. f. physiol. Chem., 1891, xv, 495.

[6] Schenck: Arch. f. d. ges. Physiol. (Pfluger's), 1894, lv, 203.

[7] MacLeod: Jour. Biol. Chem., 1909, v, 443.

phosphotungstic acid as described by Reid[8] to obtain the sugar solution. A disadvantage of all these methods lay in the fact that a certain amount of sugar was held back from the filtrate during precipitation. As a result the precipitates had to be washed sugar-free to obtain accurate estimations and this procedure required a large bulk of fluid, and considerable time was occupied in later concentrating this fluid.

Edie and Spence[9] attempted to obviate this by dialysis of the sugar from the blood into saline solution. Michaelis and Rona[10] introduced the use of colloidal iron in the presence of an electrolyte. They were able to precipitate the blood proteins completely by these reagents without absorption of sugar and therefore they could use aliquot parts of the filtrate instead of washing the precipitate. Furthermore since the iron and salt were precipitated with the protein, no necessity existed for repeated filtering. Thus this method was more rapid and simple than the older ones. Bang,[11] however, has preferred alcohol and animal charcoal as a means of obtaining the sugar filtrate.

The polarization of blood-sugar solutions has been advocated by Michaelis and Rona and their pupils. The majority of workers on the various means of quantitative sugar analysis have used one of the reduction tests. These are all based on the same underlying principle. It is known that a cupric or mercuric salt is reduced quantitatively by sugar and boiling, and if the amount of copper or mercury involved in the reaction can be estimated, the total amount of sugar in a given solution can be determined. The various tests most generally used have been gravimetric, titrimetric or colorimetric.

Allihn devised a method, modified by Pflüger[12] in which a solution of copper sulphate was boiled with an unknown sugar solution. The reduced copper was filtered, ignited and weighed as pure copper. From this figure the amount of sugar present was estimated from especially prepared tables giving the value of varying amounts of sugar in terms of copper. This test or one slightly modified has been used by Bönniger,[13] Edie and Spence, Harley,[14] MacLeod and Reid. Bertrand,[15] instead of weighing the reduced copper, dissolved it in a solution of ferric sulphate and sulphuric acid. The amount of ferrous salt so formed was determined by titration against potassium permanganate standardized with ammonium oxalate. Knowing this, the amount of copper present in the reaction was found, and the amount of sugar in an unknown solution was estimated by tables giving different copper-sugar values.

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[8] Reid: Jour. Physiol., 1896, xx, 316.

[9] Edie and Spence: Biochem. Jour., 1907, ii, 103.

[10] Michaelis and Rona: Biochem. Ztschr., 1908, vii, 329.

[11] Bang: Ztschr. f. physiol. Chem., 1910, lxv, 497.

[12] Pflüger: Arch. f. d. ges. Physiol. (Pfluger's), 1898, lxix, or 1902, xcvi, 1.

[13] Bönniger: Deutsch. med. Wchnschr., 1908, xxxiv, 780.

[14] Harley: Jour. Physiol., 1891, xii, 391.

[15] Bertrand: Quoted from Grube, Abderhalden's Handbuch der biochemischen Arbeitsmethoden, 1910, ii, 181, Urban and Schwarzenberg, Berlin and Vienna.

Bernard used Fehling's method. An alkaline copper solution was made of such strength that it was perfectly reduced on boiling with a fixed amount of sugar. In making an analysis, a part of the sugar solution was added to the copper until no further reduction occurred and the ratio which this bore to the entire solution gave information from which it was possible to discover the total quantity of sugar present. The difficulty with the test lay in the fact that the end-point was uncertain. Pavy[16] modified the reaction by making a solution of ammoniacal copper which held the reduced copper in solution but lost its blue color after complete reduction occurred. A known amount of copper was reduced by a part of the unknown sugar solution, and from this titration the entire amount of sugar present could be estimated. Kumagawa and Suto[17] have obtained good results by its use.

Bang's[18] test is on somewhat the same principle. The reduced copper is held in solution by potassium rhodamate in the presence of carbonates. In performing the test an excess of copper solution is added to the sugar, and is later reduced to a colorless solution by hydroxylamin. The amount of sugar taking part in the reaction is estimated by tables prepared from known sugar solutions and the reagents.

Instead of using copper as a reduction test, Knapp[19] selected mercuric cyanid which is reduced into metallic mercury by glucose. A solution was made which could be reduced by a known amount of sugar, and titration was carried out until no mercury salt was left in solution. Röhmann, Schenck and Liefmann and Stern[20] used the method in their studies on blood-sugar, while Tachau[21] modified it to serve as a rapid test for clinical purposes.

Wacker[22] introduced colorimetric means for determining blood-sugar. The albumin in the blood was precipitated by iron alum and sodium carbonate and the amount of sugar in an aliquot part of the filtrate was found by a sensitive color reaction between the sugar and a solution of phenylhydrazin sulphacid in concentrated sodium hydrate.

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[16] Pavy: Pavy, Chem. News, 1880, ix, 98, Abs. Ztschr. f. anal. Chem., 1880, xix, 98.

[17] Kumagawa and Suto: Salkowski's Test, Salkowski's Festschrift, 1904, p. 211, Hirschwald, Berlin.

[18] Bang: Biochem. Ztschr., 1906, ii, 271.

[19] Knapp: Quoted from Hammarsten. A text-book of physiological chemistry, 1893, 417, John Wiley & Sons, New York.

[20] Liefmann and Stern: Biochem. Ztschr., 1906, i, 299.

[21] Tachau: Deutsch. Arch. f. klin. Med., 1911, cii, 597.

[22] Wacker: Ztschr. f. physiol. Chem., 1910, lxvii, 197.

Quantitative readings were obtained by comparison of the color of unknown solutions with that of known sugar solutions mixed with the reagents. Autenrieth and Tesdorpf[23] boiled sugar solutions with Bang's copper solution, diluted the mixture to a known amount and estimated the amount of reduced copper by colorimetry. The copper solution lost color in proportion to the amount of sugar present, so that Autenrieth and Tesdorpf were able to construct a color scale of sugar values which gave accurate readings. Forschbach and Severin[24] and Borchardt and Bennigson[25] used this method satisfactorily. Reicher and Stein[26] depended on Molisch's reaction as a quantitative test. Tablets of alphanaphthol and sulphuric acid were added to a sugar solution from which the albumin was precipitated and the color changes were compared with tubes containing similar amounts of reagent and increasing amounts of sugar. The readings obtained were higher than by the other methods, which made Forschbach and Severin believe that the test was not sufficiently delicate for general use.

From this review it is seen that there are a variety of ways for estimating blood sugar, none of which are free from criticism. Difficulties have been encountered not only in precipitating the proteins from the blood but also in getting the sugar values. Constant readings, however, which have been valuable in comparative studies over long intervals of time, have been obtained by using any one method. The objections to the various tests from the point of view of the clinician have been the large amounts of blood required to conduct the analyses, the length of time for their completion, and the difficulty of technic.

Any test, to be of use in clinical studies on blood analysis, must require small amounts of blood, so that it can be repeated at will, must be accurate enough for comparative readings, and must be so simple that it can be performed by individuals without specialized training in chemical technic. Bang[27] has described a method for blood-sugar analysis which fulfills these requirements. Thannhauser and Pfitzer,[28] Götzky,[29] Bing and Windelöw,[30] Bing and Jacobsen[31] and Hirsch and Reinbach[32] have confirmed the value of this test, but have compared it with other methods in a comparatively small number of cases. In this country no systematic use of Bang's test has been reported.

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[23] Autenrieth and Tesdorpf: München. med. Wchnschr., 1910, lvii, 1780.

[24] Forschbach and Severin: Zentralbl. f. d. ges. Physiol. u. Path. d. Stoffwechs., 1911, n. f. vi, 54, 177, 665.

[25] Borchardt and Bennigson: München. med. Wchnschr., 1913, lx, 2275.

[26] Reicher and Stein: Biochem. Ztschr., 1911, xxxvii, 321.

[27] Bang: Der Blutzucker, 1913, J. F. Bergmann, Wiesbaden.

[28] Thannhauser and Pfitzer: München. med. Wchnschr., 1913, lx, 2155.

[29] Götzky: Ztschr. f. Kinderh., 1913, Orig., ix, 64.

[30] Bing and Windelöw: Ztschr. f. Kinderh., 1913, Orig., ix, 64.

[31] Bing, L., and Jakobsen, B.: Ugeskr. f. Laeger, 1913, lxxv, 1627; Abst. Jour. Am. Med. Assn., 1913, lxi, 1946.

[32] Hirsch and Reinbach: Ztschr. f. physiol. Chem., 1913, lxxxvii, 122.

This paper gives the results of a series of blood-sugar determinations made by Bang's micromethod and controlled by Bertrand's method to determine if the micromethod could be used with sufficient accuracy and sufficiently constant readings for clinical use. The technic of the two methods as used by the observer is given and the results are tabulated.

Twenty-five c.c. of blood were withdrawn from a vein according to usual technic into a small flask containing a few crystals of potassium oxalate to prevent clotting or were defibrinated by shaking. This blood was used for subsequent analysis either immediately, or after standing for a few hours to induce glycolysis. Each analysis by the two methods, however, was made at the same time. Two or three readings were made by the micromethod, and one by the Bertrand. Unfortunately enough blood for double determinations throughout was not obtained.

BERTRAND METHOD

The proteins were precipitated according to Rona and Michaelis. From 15 to 20 c.c. of blood were pipetted into a flask and were laked with 100 c.c. of distilled water; 110 c.c. of dialyzed iron solution[33] were added and 10 c.c. of saturated sodium sulphate solution. The mixture was diluted to 350 c.c. with distilled water, and was shaken for five or ten minutes. On filtering, a clear filtrate was usually obtained. In a few cases, re-filtration was necessary after the addition of a few additional cubic centimeters of the iron solution. An aliquot part of the filtrate, representing from 10 to 15 c.c. of blood was measured off, made acid with acetic acid and evaporated to 10 c.c. bulk on the water-bath. The mixture was then washed carefully into a 200 c.c. Erlenmeyer flask and the sugar estimation was conducted according to Bertrand's technic described by Grube.[34]

The sugar solution was boiled with Bertrand's two solutions for three minutes. On cooling the cuprous oxid was filtered and washed on a Gooch crucible, and was re-dissolved in a solution of ferric sulphate and sulphuric acid. The total copper present was determined by titration with potassium permanganate, and from the amount present the sugar was calculated by Bertrand's tables. From beginning to end, the entire test took from three to four hours. In the few cases in which double determinations were made, the results checked with accuracy, so that on the whole the method appeared to give constant readings and was perfectly satisfactory as a control.

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[33] The solution used was Merck's dialyzed iron (liquid 5 per cent. Fe_2O_3), the estimation was conducted according to Bertrand's technic described by Grube.[34]

[34] Grube: Abderhalden's Handbuch der Biochemischen Arbeitsmethoden, 1910, ii, 181, Urban and Schwarzenberg, Berlin and Vienna.

BANG'S MICROMETHOD

The tests were made according to Bang's original method as modified by Thannhauser and Pfitzer. The test depends on the assumption that copper is reduced by sugar without regard for the amounts present. In other words, a sugar solution from 100 mg. of blood will reduce as much copper proportionately as a solution from 100 c.c. of blood. If the microscopic amount of copper involved in the reaction can be determined the results will be as accurate as if larger amounts of the metal are used. Accordingly an albumin-free sugar solution is made from a small amount of blood and is boiled with copper solution. The cuprous oxid which is formed is held in solution by the method which Bang has described previously, and is reoxidized by iodin in the presence of potassium carbonate. Cupric carbonate and potassium bicarbonate are formed while potassium iodid is set free and can be tested for by starch as an indicator. The reaction is quantitative and extremely delicate. The technic for the test is given in detail.

The copper solution is made by dissolving 160 gm. of potassium bicarbonate in from 600 to 700 c.c. of distilled water, 100 gm. of potassium carbonate are added, 66 gm. of potassium chlorid and 100 c c. of a 4.4 per cent. copper sulphate solution ($CuSO_4 + 5H_2O$). When everything is dissolved the mixture is diluted to one liter, and is ready for use after it is allowed to stand for twenty-four hours. In making an analysis this solution is diluted with a 20 per cent. potassium chlorid solution made from 150 c.c. of saturated solution plus 70 c.c. of water. Titration is made with one-hundredth normal iodin solution prepared from 1 c.c. of 2 per cent. potassium iodate solution, 2 gm. of potassium iodid and 5 c.c. of one-tenth normal hydrochloric acid, the mixture being diluted to 50 c.c. This solution is unstable and must be renewed every three or four days.

Two or three drops of blood are placed on a small piece of thick blotting-paper, which previously has been boiled in dilute acetic acid and water to remove traces of albumin and sugar. The blotting-paper should be cut in oblongs 10 by 20 mm. and weighed before and after the blood is added. In this way the weight of blood to within 1 mg. is found. In these experiments small weighing flasks were used to hold the blood and paper. After the blood is weighed, 7 c.c. of boiling hot potassium chlorid solution is added and acidified with 2 or 3 drops of 40 per cent. acetic acid. The albumin in the blood precipitates on the blotter, while the sugar diffuses through into the solution.

After half an hour's cooling the sugar solution is transferred to a 50 c.c. Jena flask with a straight neck and is washed in by 4 c.c. of the potassium chlorid solution. In this way all the sugar in the blood is removed from the weighing flask and is ready for titration.

Three c.c. of the copper solution are now added and the mixture is boiled over a micro-burner. The heating is an important factor in the test. The solution must be warmed so that it begins to boil in between one minute and fifteen seconds and one minute and a half, and must be boiled for exactly two minutes, since the copper solution can be reduced by longer or more vigorous boiling and will give improper readings. At the end of this time the flask is immersed in cold water and titrated.

A second source of error in the determination is air oxidation of the reduced copper. During boiling this is discounted. But after cooling, air must be kept out of the solution by manipulating it under carbon dioxid gas. For this purpose Bang introduced into the neck of the flask a hooked glass tube which was connected to a carbon dioxid tank. The gas was turned on as soon as the flask was put into cold water and in this way air oxidation was prevented. Thannhauser and Pfitzer used small flasks with side-arms which were easier to handle. Similar flasks were used in these experiments.

After the two minutes' boiling, the gas is introduced and the flask is immediately cooled. This can be done in less than three seconds. A few drops of 1 or 2 per cent. soluble starch solution are now added as an indicator. The titration is made from a 1 or 2 c.c. differential pipet reading in hundredths of a cubic centimeter, and the iodin solution is added until the light blue color of the copper solution takes on the deep blue color of the starch and iodid.

Bang has tested the method carefully with known sugar solutions. The amount of iodin used by increasing amounts of sugar is so nearly constant that he has been able to make a formula for determining the amount of sugar present. The amount of iodin used in titration is divided by 2.2. This result minus 0.01 will give the milligrams of sugar present in the solution. In these experiments a special table was constructed, however, which showed the iodin readings for varying amounts of sugar from which the blood sugar readings per 100 c.c. of blood were calculated.

The entire test takes less than three-quarters of an hour, requires so little blood that it can be repeated on the same individual at will, and is very easy to perform after short practice.

The results of forty-two blood examinations are given in the accompanying table. The Bang figures represent the average of duplicate readings unless otherwise noted. All but one or two read within 0.02 of a gram of each other per 100 c.c. of blood. The amounts of blood used, varied between 50 and 120 mg., although most constant readings were obtained when from 80 to 100 mg. were taken.

RESULTS OF FORTY-TWO BLOOD EXAMINATIONS

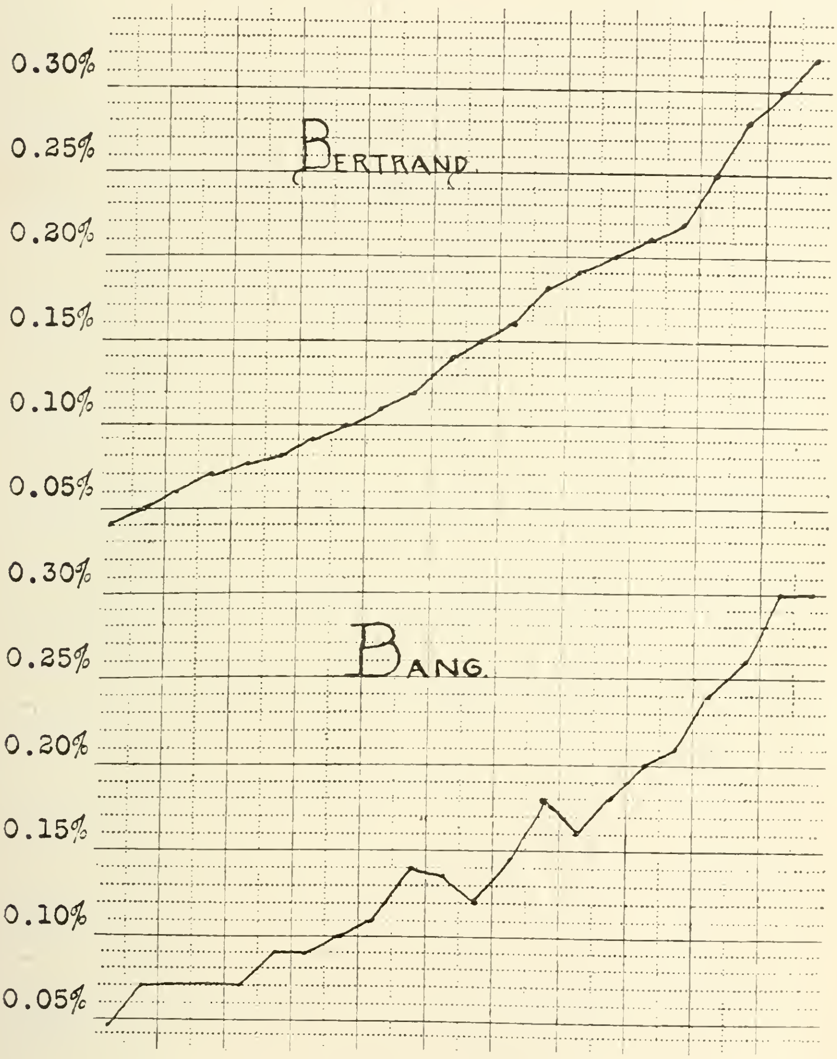
	Methods			Methods	
Blood Number	Bertrand* Per Cent.	Bang† Per Cent.	Blood Number	Bertrand* Per Cent.	Bang† Per Cent.
1	0.04	0.06	22	0.11	0.11
2	0.04	0.03	23	0.12	0.14*
3	0.05	0.06‡	24	0.14	0.12
4	0.05	0.085	25	0.14	0.15
5	0.06	0.08	26	0.15	0.12
6	0.06	0.075	27	0.16	0.145
7	0.06	0.07	28	0.16	0.15
8	0.06	0.05	29	0.18	0.17*
9	0.07	0.055	30	0.18	0.205
10	0.07	0.07	31	0.18	0.17
11	0.07	0.09	32	0.19	0.16
12	0.075	0.08	33	0.20	0.17
13	0.075	0.065	34	0.20	0.195
14	0.08	0.075	35	0.21	0.20
15	0.08	0.12	36	0.21	0.20
16	0.08	0.095	37	0.22	0.21
17	0.09	0.085	38	0.25	0.245
18	0.09	0.105	39	0.28	0.26
19	0.10	0.085	40	0.30	0.295
20	0.10	0.10	41	0.30	0.31
21	0.10	0.125	42	0.32	0.305‡

* One reading. † Two readings throughout this column except where the star (*) and double dagger (‡) occur.

If the Bertrand method is considered as the more accurate it is seen that the Bang readings were not correct. With two exceptions, however, the average Bang reading at any of the sugar concentrations encountered was within 0.03 per 100 c.c. of blood of the Bertrand. This, of course, makes the actual percentage of error large. But as the essential feature of blood sugar determinations is not so much the absolute accuracy of the figures encountered—since objections to any one method can be found—as it is the relative value in a series of comparative determinations, the Bang method is perfectly satisfactory. This is illustrated by the accompanying chart made from the average of all readings encountered at all the different concentrations.

While the curve is not absolutely parallel, yet for comparative studies it is sufficiently similar. Furthermore, as control determinations were not made by the Bertrand method, it is possible that the differences occurring may have been considerably less.

Since his first publication, Bang[35] has modified the method slightly. The copper solution is made up as before, but 1 c.c. is used instead of 3 c.c. The protein is precipitated by hydrochloric acid in potassium chlorid instead of by acetic acid. For this purpose 1,360 c.c. of saturated potassium chlorid are diluted with 640 c.c. of distilled water and 1.5 c.c. of 25 per cent. hydrochloric acid is added. Of this mixture 6.5 c.c. are used for the precipitation and added boiling hot; 6.5 c.c. are added to wash the solution into the boiling flask. Titration is done with two-hundredths normal iodin made by diluting the original



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35. Bang: Biochem. Ztschr., 1913, lvii, 300.

iodin solution to 100 c.c. instead of 50. The reagent is added until a permanent blue color appears. The amount of iodin solution used minus 12 divided by 4 gives the sugar values. In a few comparisons with Fehling's and Knapp's titration methods these results were found to be from 0.01 to 0.15 per cent. higher. This agrees with the results of Hirsch and Reinbach and Thannhauser and Pfitzer. Therefore to obtain most accurate results, Bang advises the subtraction of 0.015 from the estimated reading.

These modifications were used in a series of ten more bloods. In my experience, the results were no more accurate than by the first series. Furthermore so sharp an end-point in titration could not be obtained by the diluted iodin solution and therefore the former method is on the whole more satisfactory.

CONCLUSION

These comparative experiments show that the results obtained by the micromethod were not absolutely accurate if the results obtained by the Bertrand method are considered correct. In a series of forty-two determinations by both methods, however, in only two cases was there a difference of more than 0.03 gm. per one hundred c.c. of blood. This means that, while the absolute error was large, the relative error was slight. The curve of blood sugar determinations made from all the examinations was closely parallel. Hence for comparative and repeated studies in blood sugar estimations the micromethod is admirable. Furthermore, since the amount of blood required to make the test is so small, and the technic so simple, the method affords an excellent clinical means for the estimation of blood-sugar.

Peter Bent Burham Hospital.

BOOK REVIEWS

Diseases of the Stomach, Including Dietetic and Medicinal Treatment. By George Roe Lockwood, M.D., Professor of Clinical Medicine in the Columbia University; Attending Physician, Bellevue Hospital, New York. Pp. 624. Philadelphia and New York: Lea & Febiger, 1913.

In the preface of this book, the author states his intention, to "describe the diseases of the stomach as he has happened to see them." From this viewpoint the book is thoroughly satisfactory and deserving of hearty commendation. Moreover, such a presentation possesses a flavor of individuality and originality most welcome in an age of somewhat hackneyed disease description.

There are, however, inherent criticisms in such an endeavor applicable here as elsewhere. For the author in giving his own individual experiences fails to give critical consideration to the views of other workers in the same field, thereby omitting valuable clinical and experimental studies. This failure to include recent literature, while confessedly foreign to the author's intention, still detracts from the permanency of the book as an authoritative presentation of the subject. In other words, one misses the judicial impersonal weighing of the many problems in gastric disease, "the last word" feeling so impressively felt in standard German texts, so frequently wanting in our American. It might have been wiser had the available material, a "series of cases," been condensed and presented as a small monograph, in which form a complete survey of the field is neither expected nor desired.

The book includes instructive descriptions of gastric and duodenal ulcer (which, however, should *not* be considered together, as their pathogenesis in all probability is quite different); carcinoma (where unfortunately there *is* considerable doubt clinically and immeasureably more doubt pathologically and experimentally that they do arise from preceding ulcers); tuberculosis and syphilis, pyloric spasm, gastroptosis, neuroses, etc.

An interesting chapter on "Various Diseases and Their Gastric Relations," gives a thorough discussion of the appendix problem, and a thoroughly inadequate one of liver and gall-bladder disease; also a striking absence of reference to the relationship of diseases of women and the menstrual function; diseases of heart, kidney and metabolism; the anemias and leukemias, while chronic lead poisoning and the visceral crisis in erythemas are included. This lack of completeness, also evident in other chapters, is the most serious defect of the book.

Throughout, one feels, in the chapters concerning the more usual forms of stomach disease, the broad experience of the writer and is grateful for his conservative reasoning and sane clinical judgment. Likewise one must speak highly of the excellent plates of pathological specimens and the instructive radiograms. Also the graphic pain charts are most ingenious and refreshing and lend themselves well to class demonstration.

Several text errors have crept into an otherwise clearly typed and printed book. In the contents heading, Chapter X, "Anatomy of the Stomach," should probably be "Atony of the Stomach." On page 591, "The pancreatic fermentation may be demonstrated" for "The pancreatic ferments, etc." One would like to suggest also, that illustrative case histories be put in small type as they are elsewhere.

The Fundamental Basis of Nutrition. By Graham Lusk, Professor of Physiology, Cornell University Medical College. Cloth. Price, fifty cents. Pp. 62. New Haven: Yale University Press, 1914.

On so vast a subject as nutrition it takes a great man to write a small book. Most of the controversial questions in nutrition are touched on in this little book of 51 pages, yet without bewildering the general reader with too much technicality and discussion. Professor Lusk frankly recognizes the importance of the difference in the diets of different races and different climates. The account of the diet of Eskimos is especially interesting. The author notes that "although these people are the greatest meat eaters in the world, there is practically no gout among them. Also there is no quarreling. If two persons do not like each other, they simply move away from one another, but a blow is never struck." This point is made, no doubt, in answer to the claim so often made by vegetarians that meat stimulates the more quarrelsome side of our natures. Though aware that a man may live for long periods on low protein diet, Professor Lusk agrees with Rubner and Meltzer that the high protein requirement of Voit's standard ration is valuable as a "factor of safety" and of reserve in human life. The author's view of the Texan cow puncher's diet (page 33) is not made quite clear. There may be some misprint in this paragraph.

The author makes it clear that the American prejudice against veal has no basis in science. "In Germany light colored meats, such as chicken and veal, are considered digestible and good food for the sick. Red meat, like beef, is deemed indigestible. Veal is well cooked in Germany and greatly liked. The cooking of beef, on the contrary, is one of their unsolved problems. Ultimately the question is one of flavor. Man chooses what he likes best and, taken in moderation, what he likes best, he digests best."

The chapter on beri-beri and Funk's vitamines is especially interesting. Although clearly recognizing that polished rice is a dangerous diet, when other foods are taken in small quantities, as for example in Japan and the Philippines, the author believes that any legislation against polished rice in the United States is meddlesome and unnecessary. "The advocacy of unnecessary legislation weakens the authority of the medical profesion on sanitary matters."

In his final chapter on the monetary values of foods, the author makes a strong plea that some national authority should mark the food values on the packages in which food is sold. The manufacturer, he thinks, should be compelled by law to label his can: "This can contains x calories of which y per cent. are in proteins of grade C."

"If, through the mediums of the schools and the press everyone knew that a man of sedentary occupation required 2,500 calories and a laboring man 3,000 calories and more, no one suffering from want would spend his money for a can of tomatoes, which is little else than flavored water."

At the close of the book it is said: "Food manufacturers have recently requested a group of scientific men to meet as a board, name their own salaries and give advice. But the day of the sale of a man's scientific reputation and that of the institution with which he is connected has almost passed. The scientist has come to have sufficient altruism to believe that his services belong to all the people and not to a set of money making individuals."

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STUDIES IN THE PATHOLOGY OF THE SPLEEN *

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Many diseases of the blood are associated with striking changes in the morphology of the spleen. Often, indeed, the gross change in this organ is the dominating feature of the clinical picture. Much has been written concerning these changes, but as yet little progress has been made in the attempt to correlate changes in the spleen with clinical symptoms.

There is considerable uncertainly about our knowledge of the functions of the spleen. That this organ has important functions, however, can hardly be questioned. There is, for example, considerable evidence to show that the spleen may have a marked influence on hemolysis. It is but a step then to assume that there may exist for the spleen conditions associated with a hyperactivity of some of its functions, let us say the function of influencing hemolysis. To such a condition the term "hypersplenism" may be applied. Conversely, a condition of decreased function may be conceived to be within the range of the organ activity, a state of hyposplenism. Similar conditions have been found to exist in the metabolic activities of the thyroid and pituitary glands.

In the case of these glands, striking clinical symptoms have been grouped together into rather characteristic pictures of hyperactivity and hypo-activity of the thyroid and pituitary. If it can be shown that important clinical symptoms consistently point to a hyperfunction of the spleen, and that these symptoms disappear or are strikingly mitigated when the spleen is removed from the body, an important step will have been taken toward defining the changes in function of the spleen.

Some evidence has already accumulated to render probable the existence of these states of changed function. It will be my purpose to review briefly the more important contributions to this subject.

It was with the hope of adding some further facts to the knowledge of the pathology of the spleen that these studies were undertaken.

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More particularly, the object was to investigate closely the relation of the spleen to hemolysis, for therein lay, in my judgment, a promising path for adding to the knowledge of the etiology of various types of anemia.

One road of approach to the problem has been opened up by the studies of Faust and Tallquist.[1] They offered an explanation of the anemia caused by the *Bothriocephalus latus*. In the proglottis of the parasite there is formed a powerful hemolytic substance, cholesterin oleic acid. The oleic acid combination is split in the stomach of the host, and the free oleic acid combines with alkalies and is absorbed as a soap. In this way the soap, which injures the red blood-cells, producing hemolysis, gets into the circulation, while the cholesterin passes out unchanged in the feces.

Oleic acid belongs to the series of unsaturated fatty acids, and this work at once suggests the interesting possibility that the human organism in disease may produce hemolyzing, unsaturated fatty acids. Furthermore, it is of greatest importance to ascertain if the elaboration of such acids can be ascribed to any of the organs of the body. Our attention is at once directed to the spleen, as a fruitful possibility, more particularly because previous work in the literature indicates that the spleen is capable of influencing definitely the production of hemolysis and icterus.

It will be of interest to review briefly this evidence. Joannovics and Pick[2] have presented some important facts in their studies on toluylendiamin poisoning. They point out that a distinction must be made between the acute and chronic forms of poisoning. In the acute cases in the salt solution extract of the liver there is found an intensely working hemolysin, which is not influenced by extirpation of the spleen, and is probably produced by the immediate influence of the poison. The liver in the acute form does not show fatty changes.

In the chronic form there occurs a second hemolysin, which appears to run parallel to the fatty changes in the liver and is influenced by extirpation of the spleen. This hemolysin may be identical with that hemolytic substance which arises as a result of fatty organic changes (that is, autolysis), and consists essentially of low and higher fatty acids. The influence of splenectomy is to lessen the intensity of the fatty changes occurring in the liver. A salt solution extract of the normal liver produces practically no hemolytic action; this is also true of a normal kidney. The greater part of the intensely hemolytic

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1. Faust and Tallquist: Ueber die Ursachen der Bothriocephalusanaemia. Archiv. f. exper. Path. u. Pharmakol., 1907, lvii, 367.

2. Joannovics and Pick: Beitrag zur Kenntniss der Toluylendiaminvergiftung. Ztschr. f. exper. Path. u. Therap., 1909, xii, 185.

substance in the fatty liver is oleic acid. The abundant appearance of oleic acid and other higher fatty acids (palmitic and stearic) in the liver of toluylendiamin-poisoned dogs can be brought into agreement with the change worked in the organs by the toluylendiamin, namely, fatty metamorphosis and necrosis of the liver parenchyma.

As a result of removal of the spleen, Joannovics and Pick[2] found that dogs tolerated greater doses of the drug without showing icterus. They are of the opinion that after splenectomy there results a marked diminution in the building of higher unsaturated fatty acids in the liver of cases of subacute and chronic toluylendiamin poisoning.

In a second paper Joannovics and Pick[3] found that from the liver of acute yellow atrophy and phosphorus poisoning in man and animals, hemolysins of great activity could be obtained. Furthermore, in experimental phosphorus poisoning, these fatty acids can be found in great quantities in the blood. They suggest the explanation that the hemolytic effect of the liver extracts in these conditions is due to the unsaturated fatty acids they contain. Probably these unsaturated fatty acids are freed from their normal lecithin-like combination by the tissue destruction of the toxin or the chemical.

Joannovics,[4] in another manner, tested the influence of the spleen on hemolysis by making a fistula between the splenic vein and the left renal vein, thus diverting the splenic blood into the inferior vena cava. Animals so prepared were found to tolerate toluylendiamin better than normal animals. Joannovics gives the following conception of the action of toluylendiamin under different conditions:

In normal animals the toluylendiamin to a certain extent and the spleen to a great extent destroy the red blood-cells, and the spleen takes out the decomposition products of the red blood-cells and passes them on to the liver through the portal vein. The material for building bile-pigment is thus overabundant, and the liver makes an excessive amount of concentrated bile. There result stasis and reabsorption, causing icterus. The destruction of, red blood-cells in the spleen causes the anemia, and the changes in the liver, the icterus. With the spleen removed, we have separated the organ for destroying the red blood-corpuscles loaded with toluylendiamin. These remain in the circulation. The animal loses weight from poor nutritional conditions. Larger doses of the drug cause some destruction of red blood-cells, and there results anemia. Consequently, there comes eventually to the liver an increased amount of blood-pigment, and in the end icterus results, as in the previous case.

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[3] Joannovics and Pick: Ueber hämolytisch wirkende freie Fettsämen in der Leber bei akuter gelber Atrophia und Phosphorvergiftung, Berl. klin. Wchnschr., 1910, xx.

[4] Joannovics: Ueber die Auschaltung der Milz aus dem Pfortaderkreislauf, Separat-Abdruck aus den Verhandlung der Deutschem Pathologische Gesellschaft, v.

When a spleen-kidney vein fistula is made, the spleen remains in the body, and has the power to destroy red blood-cells and withhold them, but cannot pass them on to the liver directly. Therefore anemia is the first symptom here. The liver is not subjected to harmful decomposition products in so great amounts. Later, however, sufficient amounts of blood-pigment reach the liver to cause icterus as in the other cases. Joannovics concludes that toluylendiamin is a hemolytic substance in the sense that it only injures the red blood-cells, and does not completely destroy them. The spleen is necessary. It dissolves the injured red blood-cells and thereby subjects the liver to increased metabolism of the blood-pigments.

Pearce[5] and his collaborators have made important studies of the function of the spleen. Their attention has been directed to the broad problem of the "Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice." They have made observations of great value to the subject, and the salient points of their work must be presented before attempting to pass to the clinical aspect of the problem.

They found that after removal of the spleen, the resistance of the red blood-corpuscles increased. This was not noted before the ninth day, and did not increase after this up to seven and a half months. Furthermore, after splenectomy the injection of hemolytic serums could cause hemoglobinuria, but this was not followed by jaundice. Important was the finding that bile pigments appear in the urine of a splenectomized dog within twenty-four hours after the tying of the common bile-duct, thus ruling out the possibility that the absence of the spleen may have an effect on the elimination of bile.

The removal of the spleen was not found to change the threshold for jaundice; that is, the amount of hemoglobin necessary to be retained to produce jaundice was the same as in normal animals (0.22 gm. per kilogram). Elimination of hemoglobin by the kidney is a trifle slower than in normal animals. In other words, these investigators have shown that the absence of the spleen does not prevent the liver from forming bile-pigments from hemoglobin, or the kidneys from eliminating it. They offer lucid explanations for the occurrence of cases of jaundice without hemoglobinuria and jaundice continuing after the disappearance of a transient hemoglobinuria. In the former case there may be a gradual slow destruction of red blood-cells. "The liver removes the hemoglobin so rapidly that the concentration of hemoglobin in the serum does not reach the threshold value of the kidneys, and therefore hemoglobinuria does not occur." In the latter case, "rapid destruction of blood raises the concentration of hemoglobin in the serum so rapidly that the threshold value of the kidney is quickly exceeded and hemoglobin occurs in large amounts in the urine. When an amount of hemoglobin sufficient to reduce the concentration of the serum below the threshold value of the kidney has been removed, a considerable amount of hemoglobin may remain in the serum, and it is the slow elimination of this through the liver that causes the cholemia to continue."

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[5] Pearce, Austin and Evans: Reactions to Hemolytic Serum at Various Intervals After Splenectomy, Jour Exper. Med., 1912, xvi, 363.

In another paper, attention is called to the possibility that the failure of hemolytic serums to cause jaundice is associated with the anemia, which follows splenectomy. It might be due to the low hemoglobin content of the red blood-corpuscles.

Musser, Jr.,[6] had found that in dogs following splenectomy there occurred an anemia reaching as low as 2,800,000 and hemoglobin from 50 to 65 per cent. The animals did not return to normal for about four and a half months.

Pearce and his collaborators[5] noticed that jaundice occurred spontaneously from three and a half to four months after splenectomy, and suggested that it may be due to overregeneration of the blood leading to high red blood and hemoglobin values. An interesting correlation is made out:

> The period during which jaundice failed to follow hemolysis corresponds to the period of blood degeneration.
>
> The period of increased resistance of the red blood-cells corresponds to the period during which regeneration of the blood was occurring.
>
> The period of spontaneous jaundice corresponds to the period of more or less complete return of red blood-cells and hemoglobin to normal.

Pearce found that the mechanism of the increased resistance of the red blood-cells after splenectomy depends on the corpuscles rather than the serum. There is no increased antihemolytic property of the serum of the splenectomized animals.

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[6] Pearce, Austin and Eisenbrey: The Relation of Hemoglobinemia to Hemoglobinuria and Jaundice in Normal and Splenectomized Animals, Jour. Exper. Med., 1912, xvi, 375. Pearce, Austin and Musser: The Changes in the Blood Following Splenectomy, and their Relation to the Production of Hemolytic Jaundice, ibid., 1912, xvi, 758. Karsner and Pearce: A Study by the Methods of Immunology, of the Increased Resistance of the Red Blood-Corpuscles After Splenectomy, ibid., 1912, xvi, 769. Pearce and Austin: Change in the Endothelial Cells of the Lymph Nodes and Liver in Splenectomized Animals Receiving Hemolytic Serums, ibid., 1912, xvi, 780. Musser, J. H., Jr.: An Experimental Study of the Changes in Blood Following Splenectomy, The Archives Int. Med., 1912, ix, 592.

No explanation has been found as yet for the increased resistance of the red blood-corpuscles after splenectomy. Pearce thinks that this phenomenon is associated with the anemia, "since increased resistance of the red blood-cells is a concomitant of the regeneration of the blood which follows anemia caused by removal of the spleen."

Further studies by these workers[7] regarding the nature of the anemia following splenectomy brought out some interesting facts: The anemia makes its appearance rapidly, and reaches its height in from three to six weeks. The decrease in hemoglobin occurs more quickly and is more marked than that of the red blood-cells. Likewise, in improvement, the hemoglobin lags behind the red blood-cells. A leukocytosis is a constant accompaniment of the anemia. The normal blood-picture is not reached until from five to ten months after the splenectomy. Observation[8] of splenectomized dogs for over a year showed that the animal retained the same increased resistance of the red blood-cells and the same lessened tendency to hemolytic jaundice.

In another communication, Krumbhaar and Musser[9] point out that the anemia caused by injection of hemolytic serum was more severe in the splenectomized dog than in the normal control, and that the period of repair of the anemia was likewise longer in the splenectomized animal. Similarly the anemia following injection of sodium oleate and also resulting from hemorrhage is more severe in the splenectomized animal. Here exists an apparent paradoxical condition, in which animals with more resistant red blood-corpuscles suffer a severer and longer anemia than animals with corpuscles of normal resistance.

After injection of hemolytic serums, however, it was found that the red blood-cells of the splenectomized animal became less resistant to hypotonic salt solution, and this decreased resistance is manifested longer by the splenectomized than the normal control animals. A similar but much less striking diminished resistance of the red blood-cells occurs in the anemia caused by sodium oleate and hemorrhage.

The occurrence and persistence of this lessened resistance of the red blood-cells after the use of hemolytic serum is associated intimately with the absence of the spleen. The result of the removal of this organ is therefore, in these authors' opinion, a factor in the production of a more severe and prolonged anemia which hemolytic

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7. Musser, J. H., Jr., and Krumbhaar, E. B.: The Blood-Pictures at Various Periods After Splenectomy, Jour. Exper. Med., 1913, xviii, 487.

8. Pearce and Peet: The Effect of Hemolytic Serum in Splenectomized Dogs, Jour. Exper. Med., 1913, xviii, 494.

9. Krumbhaar, E. B., and Musser, J. H., Jr.: The Relation of the Spleen to Blood Destruction, Jour. Exper. Med., 1913, xviii, 665.

serums cause in splenectomized animals. The hemolytic serum starts the acute anemia, but the absence of the spleen is instrumental in increasing the severity and in either prolonging its course or delaying the process of repair.

Krumbhaar and Musser think that the phenomena associated with absence of the spleen (1) increased resistance of the red blood-cells and (2) decreased tendency to jaundice, are closely related to the anemia which follows splenectomy. The anemia itself depends on some other factor, unknown at present, which is active when the spleen is removed from the body.

Hemolytic serums cause a constant leukocytosis of the polymorphonuclear neutrophil type. This is usually more marked in the splenectomized animal. Likewise a persistent increase in the eosinophils and a moderate increase in the lymphocytes occurs in the splenectomized animal (the latter also occurs in the control animals).

Eppinger[10] states that there are many facts known which speak for the occurrence of hemolysis in disease. In spite of the fact that we have accurate ways of judging, often the symptoms of increased blood destruction escape our notice. The signs of continued hemolysis, he continues, are (a) histological in blood, polychromatosis, and anisocytosis; granulation of the red blood-cells; (b) chemically, in blood, increased color-index, and (c) in urine, urobilinuria. In spite of these criteria, one can obtain only a fair idea of the intensity of the process.

Also there arises the question, in each type of anemia, whether the cause is a diminished production of red blood-cells or an increased destruction. Likewise, in polycythemia doubt exists as to whether there is an increased formation or diminished destruction of blood.

A consideration of the fate of the red blood-corpuscle points out a method of studying the amount of destruction of blood taking place in disease. The oldest of the red blood-corpuscles give up their hematin in the liver-spleen circulation. After separation of the iron, bilirubin (and biliverdin) are formed. Bacteria in the intestines reduce these to urobilinogen. An exact measure of the transformation of hematin would be the quantitative determination of the bile-pigments (since it is assumed that from a molecule of hematin a molecule of bilirubin results). Since such a procedure cannot be done clinically, however, there remains the choice of determining the urobilin in the feces.

Eppinger and Charnas report clinical studies, using a colorimetric method for determining the urobilin in feces, which was devised by the latter. Thus it was found that in hemolytic icterus, pernicious anemia, pneumonia, lead-poisoning and malaria, high urobilin values obtained. Low values were found in Addison's disease and carcinoma of the liver.

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[10] Eppinger, H.: Zur Pathologie der Milzfunktion, Berl. klin. Wchnschr., 1913, L, 1509, 1572.

In pernicious anemia and hemolytic icterus there is at work some influence causing an increased destruction of blood.

Eppinger[10] had a series of splenectomies performed in the following clinical conditions: two cases of hemolytic icterus; two cases of pernicious anemia; three cases of probable Banti's disease; two cases of hypertrophic cirrhosis of the liver, and one case of icterus catarrhalis, threatening to pass into acute yellow atrophy of the liver.

All showed remarkable improvement after splenectomy, without any immediate deaths. The anemia improved strikingly, the icterus cleared up and the urobilin values in the stools returned to their normal values. (For details see Eppinger's papers.[10]) The experimental data. about to be presented, the clinical observations before and after splenectomy, the influence of the removal of the spleen on the output of bile in animals (Pugliese), and on the urobilin output in man, speak for a hemolytic function of the spleen.

Eppinger[11] reports that the histological picture in extirpated spleens of pernicious anemia, and hemolytic icterus, showed an enormous hyperemia in the spleen sinuses. In contrast, the spleens of morbus Banti, and hypertrophic liver cirrhosis presented the so-called *fibroadenie.* Eppinger suggests, in his paper, the possibility that the *fibroadenie* is a kind of healing process against the increased hemolytic activity of the spleen. Probably, he continues, pernicious anemia, hemolytic icterus and hypertrophic liver cirrhosis are all referable to an increased splenic hemolytic activity.

Pribram[12] found that stasis of blood in the spleen, as caused by chronic passive congestion, is associated with increased destruction of red blood-cells. He showed that urobilinuria could be readily caused by inducing congestion of the spleen. Also, the stasis in the inferior vena cava, such as associated with uncompensated valvular lesions, is accompanied by marked urobilinuria. Pribram calls attention to cases in the literature of isolated stasis in the spleen with increased destruction of red blood-cells and consequent anemia and urobilinuria.

It appears, therefore, from this brief perusal of the literature, that there is sufficient evidence to warrant attributing to the spleen a very important influence in diseases and experimental conditions associated with hemolysis. The problem before us appeared to be to ascertain in the diseases associated with hemolysis whether or not unsaturated fatty acids could be demonstrated in the blood, and, if so, in what relation to the character of the disease. The blood appeared to be the most promising and practical medium for the study of the unsaturated fatty acids. Since cholesterin also contains unsaturated fatty acids, it was deemed advisable to separate from the total lipoids and fats of the blood, the cholesterin and cholesterin esters, before determining the iodin number (*Jodzahl*), which was to serve as our index of the unsaturated fatty acids.

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[11] Eppinger, H.: Zur Pathologie der Milzfunktion, München. med. Wchnschr., 1913, No. 35, p. 1952.

[12] Pribram: Hypersplenische Hämophthisen und Stauungsmilz, Wien. klin. Wchnschr., 1913, xxvi, 1607.

The experiments of various authors have shown that the unsaturated fatty acids possess hemolytic properties. The most common of this class of substances which occurs in the human body and in animals is oleic acid. Its iodin-combining power is 90 (that is, the iodin number). Other acids of this series, more unsaturated than the oleic acid and with a higher iodin number, also occur in the body.

A property which has been assigned to unsaturated fatty acid is the power of hemolyzing red blood-cells. As saturated fatty acids, such as palmitic acid, possess only feeble hemolytic power, this property was preferred to the unsaturated carbon atoms.

McPhedran[13] has made a study of the hemolytic properties of fatty acids. His attention was directed particularly toward the relation of the activity of the hemolysis to the degree of unsaturation of the acids. In brief, he found that hemolysis was not more active in proportion to the degree to which the acids were unsaturated. Furthermore, the hemolytic activity of these acids was not diminished when they are saturated by halogens; but saturation of the combination by hydroxyl groups did greatly diminish their hemolytic activity.

McPhedran, in conclusion, states his belief that sufficient evidence does not exist to refer toxic hemolysis, in disease in various conditions, such as phosphorus and toluylendiamin poisoning, to the liberation of specially hemolytic fatty acids from fatty complexes of disintegrating cells. He asserts that neither such fatty acids nor complexes from which they could be obtained have been found in the organs, that no evidence exists for the occurrence in disease of fatty acids more toxic than oleic acid. The latter, he points out, is being set free by hydrolysis from fat in health.

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[13] McPhedran: Hemolytic Properties of Fatty Acids and Their Relation to the Causation of Toxic Hemolysis and Pernicious Anemia, Jour. Exper. Med., 1913, xviii, 527.

Lamar,[14] however, in studying the hemolytic properties of linoleic and linolenic acids, both more unsaturated acids than oleic acid, found that in the form of their sodium salts they dissolve pneumococci more rapidly in higher dilution than sodium oleate. Their intensity of action on the cocci is directly proportional to the degree of unsaturation of the acid.

The hemolytic and bacteriolytic activity of the soaps of the unsaturated fatty acids, he concludes, is probably due in part to their avidity for protein and not wholly to their ability to dissolve lipoids.

Meyerstein and others have studied the agents existing in the serum, which have a protective action against hemolysis. Included in this class are the neutral fats, the lipoids and the cholesterin bodies. It was therefore considered of importance to study, in association with the unsaturated fatty acids, also the total fats and lipoids and the free and bound cholesterin. It was hoped that some relation of these substances to the variations in the intensity of the hemolytic process might be found. This phase of the problem will be discussed later.

Our problem, then, was to study, if under different pathological conditions, the iodin number showed differences which could be referred to the hemolytic condition, and to changes in the spleen function.

CLINICAL AND EXPERIMENTAL INVESTIGATIONS

Blood was removed from the patient's veins by trochar, and rapidly defibrinated. One hundred c.c. were measured in a carefully graduated pipet, and delivered into a 1,000 c.c. measuring-cylinder. The flask was then filled with 95 per cent. alcohol to the 1,000 c.c. mark and the mixture stirred well and allowed to stand for twenty-four hours. The precipitated protein of the blood was then filtered into a larger Soxhlet filter, washed with alcohol, and dried at a temperature of 37 C. It was then extracted with petroleum ether (boiling-point from 37 to 40 C.) for three days.

The alcoholic filtrate was then evaporated to a suitable volume and extracted with petroleum ether for from twenty-four to forty-eight hours. The two petroleum ether extracts were then united and evaporated to dryness in a weighed glass dish, brought to a constant weight by drying and weighed. Thus the total fats plus lipoids in the blood were obtained.

The weighed contents of the dish were then treated repeatedly with hot 95 per cent. alcohol, and the cholesterin free and bound

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14. Lamar: Chemico-Immunological Studies in Localized Infections, Jour. Exper. Med., 1911, xiv, 256.

TABLE 1.—EFFECT ON UNSATURATED FATTY ACIDS OF REMOVAL OF THE SPLEEN

Animal		Condition	Total Fats in 1,000 c.c. Blood, gm.	Cholesterin in 1,000 c.c. Blood, gm.	Cholesterin Esters in 1,000 c.c. Blood, gm.	Iodin Number	Remarks
Dog 1		Normal	5.810	0.669	0.428	94.5	
		Ten days after splenectomy	9.556	0.890	0.322	15.2	
Dog 2		Normal	4.825	0.380	0.436	104.3	
		Twelve days after splenectomy	7.208	0.938	0.384	21.5	
Dog 3		Normal	7.064	0.521	0.247	113.3	
		Fourteen days after splenectomy	8.411	0.614	0.576	8.9	
Dog 4		Normal	6.601	0.572	0.306	110.9	
		Fourteen days after splenectomy	9.085	0.617	0.390	12.3	
		Two months after splenectomy	8.328	0.625	0.309	60.1	
Dog 5		After splenectomy and complete depancreatectomy	11.033	0.765	0.355	0.0	Marked glycosuria.
Dog 6		After splenectomy and complete depancreatectomy	7.444	0.406	0.239	0.0	Marked glycosuria.
Dog 7		Normal dog poisoned with toluylendiamin	16.792	1.392	0.297	146.0	
Dog 8		Splenectomized dog poisoned with toluylendiamin	9.585	2.967	0.122	127.0	
Dog 9	Serum	Normal	4.449	0.120	0.132	101.2	
		After splenectomy	9.815	0.318	0.348	26.0	
	Red blood-cells	Normal	3.883	0.393	0.048	53.8	
		After splenectomy	6.377	0.192	0.262	53.3	

and the unsaturated fatty acids went into solution. It was then filtered. In the filtrate, the cholesterin and cholesterin esters were determined gravimetrically after precipitation with digitonin solution, as given in the method by Hermann and Neumann,[15] and the unsaturated fatty acids by the Hüblschen iodin number method.[16] A qualitative reaction for the presence of cholesterin in the blood was employed as described by Neumann and Hermann. This consisted in taking 1 c.c. of blood, and adding to it 10 c.c. of concentrated sulphuric acid and a few drops of chloroform. It is then shaken and allowed to stand for twenty-four hours. If cholesterin is present the chloroform takes on a red-violet to purple color in a short time, certainly in a few hours. If no cholesterin is present, the chloroform remains colorless.

At the same time that clinical cases were investigated, experiments were performed on dogs to ascertain what effect total removal of the spleen would have on the general fat-content of the blood, in particular, the unsaturated fatty acids. Here the most complete condition of hyposplenism could be produced, and the results might, it was hoped, give us some light on the findings in the various pathological conditions, studied clinically.

These results are presented in Table 1, and discussed first, before the results in the clinical cases are presented.

THE RESULTS OF SPLENECTOMY

Removal of the spleen produces some very striking changes in the fat and lipoid content of the blood. Following splenectomy, there is an increase in total fats and lipoids, an increase in cholesterin and a decided change in the unsaturated fatty acids, as expressed by the iodin number. This is reduced to a very low value. The cholesterin esters show no constant change.

After splenectomy of two months' duration the iodin number increases again, but does not quite return to normal.

In the experiment with Dog 9, the red blood-corpuscles and serum were studied independently. A large amount of blood was centrifugalized, and the serum pipetted off. The red blood-corpuscles were then centrifugalized with normal saline repeatedly to remove all traces of serum, and the serum and red blood-cells studied independently. The findings show that the total fats increase in both the serum and the red blood-cells, after splenectomy. The increase in cholesterin occurs in the serum, while the red blood-corpuscles lose in their cholesterin content. The unsaturated fatty acids' value is markedly diminished in the serum.

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[15] Hermann and Neumann: Uber der Lipoidgehalt der Blutes normales und schwangerer Frauen sowie neugeborener Kinder, Biochem. Ztschr., 1912, No. 43, p. 47.

[16] Abderhalden: Handbuch der Biochemie Arbeits Methoden, 19, ii, 208.

The results of splenectomy indicate, then, that the spleen has some important influence on the unsaturated fatty acids circulating in the blood as well as the total fats. These unsaturated fatty acids are hemolytic. Here there seems to be established a direct relationship of the spleen to hemolysis, through the mediation of its regulation of the unsaturated fatty acids in the blood. The removal of the spleen reduces this hemolytic agent to a minimum, and therein may lie the explanation of the great benefit of removal of this organ in conditions associated with hemolysis, such as pernicious anemia, catarrhal jaundice, cirrhosis of the liver and hemolytic jaundice.

The increase in total fats and cholesterin in the serum is important. It is well known that these substances, especially cholesterin, are antihemolytic. Cholesterin will inhibit the hemolytic action of the cobra hemolysin and saponin. It might be, therefore, that its increase in the serum after splenectomy is the true explanation of the increased resistance of splenectomized animals to hemolytic agents, and also responsible for the increased resistance of the red blood-cells.

Complete removal of the pancreas shortly after splenectomy brought out some interesting points, as seen in the cases of Dogs 5 and 6. Here the striking result was the reduction of the unsaturated fatty acids to nil. Both dogs were intensely glycosuric.

The effect of toluylendiamin poisoning on a normal and a splenectomized dog was tried. The analyses showed in both cases a considerable increase in the total fats in the blood, but strikingly less in the splenectomized dog. This is of interest in relation to the statements in the literature that removal of the spleen greatly lessens the fatty metamorphosis following poisons associated with hemolysis. The very striking increase in the cholesterin in the blood in the splenectomized animal, amounting to a cholesterinemia, is significant. It may be indeed of importance in protecting the splenectomized animal against the hemolytic effects of the unsaturated fatty acids developed in the liver by this poison. There is no striking difference in the values of the unsaturated fatty acids.

As far as the blood is concerned, then, there seem to be constant findings in this condition of experimentally produced asplenism: namely, increase in the total fats and lipoids, increase in the cholesterin, and very low iodin values for the unsaturated fatty acids; that is, a diminished iodin number.

No clinical symptom-complex of hyposplenism has been made out as in the case of hypopituitarism and hypothyroidism. The existence

TABLE 2.—Results of Analysis in Various Clinical Conditions

Disease and No. of Case	Qualitative Cholesterin Test	Total Fats in 1,000 c.c. of Blood, gm.	Cholesterin in 1,000 c.c. of Blood, gm.	Cholesterin Esters in 1,000 c.c. of Blood, gm.	Iodin Number	Remarks
Normal, 1	0	5.380	0.764	0.528	90	
Normal, 2	0	5.900	0.864	0.575	79	
Pernicious anemia, 1	+	7.347	0.564	0.722	188	Diagnosis in all these cases confirmed at necropsy.
Pernicious anemia, 2	+	8.402	0.318	0.009	213	
Pernicious anemia, 3	+	9.379	0.144	0.047	273	
Hemolytic jaundice, 1	+	5.434	0.491	0.415	326	Present for seven years.
Hemolytic jaundice, 2	0	6.327	0.854	0.313	258	Present for three years.
Cirrhosis of liver, 1	+	9.346	0.906	0.314	125	Large liver; medium large spleen; jaundice.
Cirrhosis of liver, 2. (In coma hepaticum.)	+	3.945	0.303	0.131	309	Large liver; medium large spleen; jaundice.
Cirrhosis of liver, 3	+	7.361	0.563	0.124	81.8	Atrophic form; no jaundice.
Cirrhosis of liver, 4	+	4.959	0.336	0.131	84.8	Atrophic form; no jaundice.
Cirrhosis of liver, 5	+	6.527	1.115	0.161	82.3	Atrophic form; no jaundice.
Cirrhosis of liver, 6	0	4.350	0.285	0.077	265.0	Ascites; marked jaundice.
Cirrhosis of liver, 7	+	4.061	0.191	0.361	117.6	
Catarrhal jaundice, 1	+	5.700	0.504	0.231	187	Incomplete obstruction to outflow of bile.

Catarrhal jaundice, 2........	+	5.400	0.391	0.440	123	Incomplete obstruction to outflow of bile.
Catarrhal jaundice, 3. (After	+	5.594	0.483	0.675	121.6	Large spleen; jaundice.
removal of spleen.)	+	7.205	0.861	0.633	83.4	Jaundice cleared up.
Catarrhal jaundice, 4........	+	7.661	0.674	0.480	63.3	
Catarrhal jaundice, 5........	+	10.060	0.986	0.729	83.7	
Secondary anemia, 1.........	+	6.395	0.715	0.524	36.9	Carcinoma of stomach; symptoms of stenosis.
Secondary anemia, 2.........	+	6.966	0.377	0.441	101.6	Carcinoma of esophagus.
Obstructive jaundice, 1.......	+	10.630	1.068	0.275	88	Complete obstruction to common duct for eight months. (Stone in common duct.)
Obstructive jaundice, 2.......	+	7.919	0.613	0.494	139	Complete obstruction for four months; carcinoma.
Nephritis, 1	—	8.243	0.465	0.385	22.3	Small granular kidney; uremia, coma.
Nephritis, 2	+	15.895	1.260	0.069	69.0	Small granular kidney.
Bronchiectasis, 1	+	5.440	0.554	0.143	169.6	Jaundice.
Lobar pneumonia, 2..........	+	7.561	0.662	0.110	28.5	Jaundice; before cirrhosis.
Malaria	+	8.370	0.745	0.143	29.9	Tertian parasite in blood. Enlarged spleen; several previous attacks.
Hemochromatosis, 1	+	9.765	1.105	0.797	56.5	Pigmentation glycosuria.
Hemochromatosis, 2	+	6.278	0.910	0.714	30.2	Pigmentation glycosuria.
Syphilis, 1	+	4.648	0.711	0.121	97.2	Jaundice, tertiary stage.
Polycythemia, 2	+	5.930	1.094	0.079	272.9	
Purpura, 3	+	8.498	0.861	0.633	224.5	
Cholangitis, 4...............	+	5.968	0.485	0.077	123.3	Jaundice.

of a condition of diminished function of the spleen in clinical pathology has not been shown. It is true that the simple fact that total removal of the spleen is not of serious consequence to a human being has rather drawn the attention of observers away from this problem.

ANALYSIS OF VARIOUS CLINICAL CONDITIONS

It is my purpose next to consider the results of analysis obtained in various clinical conditions. The figures are given in Table 2 and the cases, as far as possible, brought together into groups.

The values in two normal cases are first given for comparison.

Pernicious Anemia.—The three cases presented here were typical in their clinical findings and blood-picture of pernicious anemia.

In each case the total fats were increased above normal, while the free cholesterin was decreased to varying degrees. The striking finding, however, was the very high values for the iodin number. It is significant that the blood with the highest iodin number showed the lowest value in free cholesterin. Evidently, unsaturated fatty acids of very high iodin-combining power are present in the blood of pernicious anemia. The thought at once occurs that the presence of such highly unsaturated fatty acids in the blood may, through their hemolytic powers, be responsible for the anemia in this condition. Furthermore, as very low values for the iodin number are obtained in the blood of splenectomized animals, it may be permitted for the moment to consider that in pernicious anemia a condition of hypersplenism may exist. A full consideration of this hypothesis will be given in the general discussion, reserved for the conclusion of this paper.

Hemolytic Icterus. — In this group two cases are given. The striking feature of the analyses are the very high iodin-combining values for the unsaturated fatty acids. Here again the higher iodin number is associated with the lower free cholesterin value.

Cirrhosis of Liver.—The cases presented here show interesting variations.

Case 1 presented an enlarged liver, a moderately enlarged spleen and marked jaundice. There was an alcoholic history. Opportunity was given for observation of the case in two stages, first when the patient was feeling relatively well, and second when in a condition of coma hepaticum. The most striking result of the analyses is the considerable change in the value of the iodin number. Already of high value, it increased remarkably when the patient went into a condition suggesting acute yellow atrophy. At the same time the total fats and cholesterin, already high, decreased much in their values.

Cases 3, 4 and 5 show low values for the iodin number. They were cases of typical atrophic cirrhosis. It is interesting that none showed icterus.

Case 6 presented marked icterus and ascites. Here the iodin number is extremely high.

Case 7 likewise showed a high iodin number.

Reviewing the cases as a whole, the most striking feature is that the cases which are associated with icterus have a high iodin number, while those without this symptom present lower values.

Analyses to be given later in a case of mechanical obstruction to the common bile-duct will show that these high values of the iodin number are not due to the absorption of bile.

Catarrhal Jaundice.—These cases present some interesting features. In general, the total fats have from about normal to slightly increased values; but the iodin number values are higher than normal and the free cholesterin values less than normal. Case 3 is especially interesting. In this case the spleen was removed, with the result that the icterus cleared up and also the urobilinuria. It is a striking fact that the value of the iodin number decreased from 123 to 83 (about normal). As in the animal experiments, coincident with this, the total fats and cholesterin increased in the blood.

Case 5 shows a low value of the iodin number with a considerable increase in the total fats and cholesterin.

Eppinger calls attention to some interesting points relating to this group. He is of the opinion that there are often cases of apparently harmless icterus which come on in the course of a gastro-intestinal intoxication. Relatively early there appears an enlargement of the liver, with rise in temperature. In contrast to the usual icterus catarrhalis the stools remain cholic. In the urine there is much urobilin and urobilinogen, but not much bile-pigment. These patients often die suddenly with signs of acute yellow atrophy of the liver. Eppinger had a splenectomy performed in a case of this kind, in which there was an appearance of oncoming acute yellow atrophy. The jaundice cleared up and the patient lived.

Many such cases present signs of cholelithiasis. It is remarkable that in spite of the patency of the bile-passages, attacks of pain suggesting gall-stone colic can occur. At the time of seizure of pain, enlargement of the liver was made out.

Secondary Anemia.—A case of secondary anemia associated with carcinoma of the stomach is given in this group. It will be seen that the value of the iodin number is low, in striking contrast to that shown in the pernicious anemia cases. The total fats and cholesterin values are approximately normal.

A second case of carcinoma of the esophagus shows a somewhat higher iodin number, with the cholesterin value below normal.

Icterus Gravis.—In Case 1, obstruction by stone, the iodin number value is about normal, while the total fats and cholesterin are markedly increased. Case 2 is one of complete obstruction due to carcinoma with mechanical stasis of the bile outflow. Here the iodin number is somewhat increased. The findings in this case indicate that the high values of the iodin number in the previous cases are not due to simple retention of bile.

Nephritis.—Two cases are reported in this group.

Case 1 was an example of uremic coma. The value of the iodin number was very low.

In Case 2 there was a small contracted kidney, a case of genuine *Schrumpfniere*. The total fats were considerably increased as well as the cholesterin, while the iodin number was below the normal value.

Pulmonary.—Case 1 was one of bronchiectasis associated with icterus, of a hemolytic type. The value of the iodin number is above normal.

Case 2 was a case of lobar pneumonia associated with icterus. Here the value of the iodin number was below normal. The blood was taken early in the disease, before the crisis.

Malaria.—A case is here given in which the tertian parasite was found in the blood. The spleen was enlarged. There was no jaundice. The value of the iodin number is below normal.

Hemochromatosis.—Two cases were examined in this group. Both showed increase in the total fats, and cholesterin in the blood. In both cases there were low values found for the iodin number. Neither case showed evidences of jaundice, but both presented glycosuria.

Syphilis (General).—A case is given of syphilis, associated with jaundice. The iodin number showed practically a normal value.

Polycythemia.—A typical case of this disease presented unusual interest. The red blood-cells were considerably over 6,000,000 and the hemoglobin correspondingly high. A very high value of the iodin number was found, and also an increased cholesterin content in the blood. In view of our previous findings of high values for the iodin number associated with evidences of hemolysis, the results in this case might be construed to suggest that in the condition of polycythemia increased destruction of red cells is taking place, rather than a diminished destruction.

Purpura.—The findings in a case of purpura are interesting. Here a very high value for the iodin number was obtained, together with an increase in the total fats in the blood.

Diabetes.—A series of cases associated with lipemia were examined. The detailed results will be presented in another paper. Suffice it to say here that the values of the iodin number were considerably below normal, although the total fats were enormously increased, up to 107.804 gm. per thousand cubic centimeters of blood in one case.

These cases demonstrate that the high values of the iodin number are not dependent on the content of the serum in the total fats.

GENERAL DISCUSSION

The evidence resulting from this study of the function of the spleen seems to demonstrate a parallelism between high values for the iodin number in the blood and hemolysis. Clinical conditions in which anemia and icterus of the hemolytic type are striking symptoms of the disease are associated with high iodin-combining values for the unsaturated fatty acids. This is especially characteristic of the clinical groups, pernicious anemia, hemolytic jaundice and catarrhal jaundice.

The fact that removal of the spleen causes the values of the iodin number in the blood to sink to a level much below normal would suggest that the spleen is concerned in an intimate manner with the symptom-complex of hemolysis, icterus and anemia, in disease. This belief is strengthened by the influence that removal of the spleen has been found to have on the output of the bile-pigment in animals and urobilin in man. The results which Eppinger reports in a series of clinical conditions show that removal of the spleen produces a beneficial effect on the symptoms which are associated with hemolysis, that is, anemia and icterus. One case in our series of catarrhal jaundice likewise shows that splenectomy causes the jaundice to clear up and the patient's condition to improve. Several other authors have reported improvement as a result of splenectomy. A. von Decastello[17] in a case of pernicious anemia, Pollitzer[18] in a case of hemolytic type of splenomegaly, G. Klemperer and Hirschfeld[19] in pernicious anemia, F. Nidd[20] in a case of Banti's disease, and Giffin[21] in a report of twenty-seven cases of splenectomy.

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[17] Von Decastello: Splenectomie bei pernizioser Anämie, Wien. klin. Wchnschr., 1913, xxvi, 951.

[18] Pollitzer: Ein Fall von Splenomegalia hemolytica, Wien. klin. Wchnschr., 1913, xxvi, 952.

[19] Klemperer and Hirschfeld: Milzexstirpation bei pernizioser Anämie, Therap. d. Gegenw., 1913, No. 19.

[20] Nidd: Banti's Disease Cured by Splenectomy, Proc. Roy. Soc. of Med., 1913, vi, Surg. Sect., p. 225.

[21] Giffin: Clinical Observations Concerning Twenty-Seven Cases of Splenectomy, Am. Jour. Med. Sc., 1913, cxvl, 781.

The picture in these clinical conditions associated with a high iodin number is so diametrically opposite to that following removal of the spleen, as far as the features of hemolysis are concerned, that I am tempted to make a provisional distinction between the two. This is presented only as a helpful schema in the attempt to correlate the functions of the spleen with clinical symptoms. Indeed, the fact that substances which produce hemolysis fail to cause icterus when the spleen is extirpated, and likewise that splenectomy apparently benefits certain types of pernicious anemia, point strongly to the spleen as the center of the disease in pernicious anemia.

INFLUENCE OF REMOVAL OF THE SPLEEN ON HEMOLYSIS, ANEMIA AND ICTERUS

Hypersplenism:	Asplenism:
High iodin number.	Low iodin number.
Hemolysis.	Greater resistance to hemolyzing agents.
Anemia.	Improvement of anemia by splenectomy.*
Icterus.	Difficulty in producing jaundice by hemolytic agents.

* Pearce and his coworkers have shown that splenectomy causes in dogs a definite and prolonged anemia. The reported improvements following removal of the spleen in certain types of anemia in human beings seems to raise here a perplexing contradiction. It may be that removal of a spleen which has once been the seat of a pathological process is followed by entirely different effects from those which attend the removal of a normal spleen. More light is needed on this point.

It has been extremely puzzling to understand why, after removal of the spleen, the resistance of the red blood-corpuscles should be increased. No satisfactory explanation has yet been presented.

Removal of the spleen in our series of animal experiments has been found, in every case, to be associated with increase in the total fats and cholesterin in the blood. These substances are protection against hemolytic agents, and their increased quantity in the serum may be helpful in preventing the action of hemolysins. The red blood-corpuscles themselves show an increase in the total fats and lipoids after splenectomy, and to this may be due the increased resistance to hypotonic salt solution which they show *in vitro* when every trace of serum has been removed by centrifugalizing. Furthermore, the case of catarrhal jaundice in which the spleen was removed showed the same increase in the total fats and lipoids and cholesterin in the blood. This was parallel with the improvement of the clinical symptoms.

Splenectomy then would appear to reduce the iodin number from a high value, which was found to be characteristic of conditions associated with hemolysis, to a very low value, and at the same time to increase the antihemolytic substance in the blood.

In the conditions in which hemolytic processes are at work, the liver plays an important rôle. It may become enlarged, its border hard and sharp and tender to palpation. Icterus and urobilinuria may follow. If there be added to this the insult of the toxins of infectious diseases or abnormal use of alcohol, then a true cirrhosis of the liver may develop rapidly.

It seems probable then that a process primarily manifesting itself in a disturbed function of the spleen can produce change in the liver of various grades up to the so-called hypertrophic cirrhosis. Likewise, a primary liver disturbance such as alcoholic cirrhosis can be influenced by the amount of hemolytic activity that the spleen takes in the process. This is brought out by the study of two cases:

1. First was a case of cirrhosis, with an alcoholic history, relatively large liver and spleen with marked ascites, icterus, anemia and bile-pigments and urobilin in the urine. The iodin number was 265.

2. The second case was one of typical Laennec cirrhosis, with no icterus, and marked ascites. The iodin number was 84.8. The former is the picture of the so-called hypertrophic type, the latter of the atrophic type.

Eppinger considers hypertrophic cirrhosis as a combined disease in which the spleen takes an outspoken part, and is also of the view that combinations of hypertrophic cirrhosis and pernicious anemia exist.

The diseases of the "hepato-lienalic system are then polyvalent in character." The symptom-complex resulting depends on whether the liver or the spleen is most involved in the reaction. The many-sided symptoms of diseases in this category are in accord with this idea.

The following conception of the mechanism of the change produced in the diseases characterized by hemolysis seems justified from this study:

In pernicious anemia and hemolytic jaundice, and to a certain extent in some types of cirrhosis of the liver, increased hemolysis is taking place. Coincident with this the blood is found to show an abnormally high iodin-combining power, indicating the presence of highly unsaturated fatty acids. The occurrence of these acids is closely related to the activity of the spleen, since removal of the spleen lessens their value in the blood, and greatly benefits the hemolytic condition.

It is therefore possible to correlate with the increased hemolytic function of the spleen definite clinical symptoms; hemolysis, anemia, icterus. When such symptoms are present, and the blood shows high values for the iodin number, then it means that the spleen is abnormally active in influencing hemolysis, and its removal appears to be indicated.

What causes the spleen to take on this function of increased hemolytic activity is not clear at present. Further investigation must determine whether there is some fundamental condition at work bringing about this activity on the part of the spleen, or whether the process is primarily a disease of the spleen. The results of the qualitative cholesterin test are of little value, since the test has been positive with very low cholesterin value, and again negative with high cholesterin values. No quantitative interpretation can be given to this test.

CONCLUSIONS

1. Following splenectomy, there occurs an increase in the total fats and cholesterin in the blood, and also a reduction in the value of the iodin number (that is, a lower iodin number).

2. In clinical conditions associated with hemolysis, pernicious anemia, hemolytic jaundice, catarrhal jaundice and some forms of cirrhosis of the liver, there is found a decided increase in the value of the iodin number.

3. There appears to be a parallelism between highly unsaturated fatty acids in the blood and hemolysis.

4. The occurrence of these highly unsaturated fatty acids is intimately dependent on the activity of the spleen, for removal of that organ reduces the iodin-combining value of the unsaturated fatty acids and renders the production of icterus by hemolyzing agents more difficult. Also it greatly improves clinical conditions in which hemolysis is at work.

5. This is interpreted to mean that the spleen may be the central point in many diseases in which hemolysis and anemia are active, especially in pernicious anemia. This organ may be conceived to take on an increased function as regards its hemolytic activities, resulting in hemolysis, anemia and icterus.

6. The condition of asplenism presents in some of its features a diametrically opposite picture. Animals in this condition are more resistant to hemolyzing agents, and icterus is much more difficult to produce.

7. The increase in the total fats and lipoids following splenectomy may explain the increased resistance to hemolysins and the increased resistance of the red blood-cells found to accompany the condition of asplenism. These substances have definite antihemolytic properties.

8. In pernicious anemia, hemolytic jaundice, some types of catarrhal jaundice and cirrhosis of the liver, the hyperactive spleen unfavorably influences the anemia, through its regulation of the highly hemolytic unsaturated fatty acids in the blood. Its removal appears therefore to be indicated.

9. Splenectomy of itself, besides influencing the hemolytic unsaturated fatty acids, raises the percentage of antihemolytic substances in the blood, that is, the total fats and cholesterin.

10. The fact that total removal of the spleen is not followed by any symptoms dangerous to the life of the patient has probably drawn attention away from the consideration that states of diminished function of this organ may exist.

Note.—This work was undertaken at the suggestion of Dozent Hans Eppinger, and carried out in the wards and chemical laboratories of the First Medical Clinic (Hofrat Karl von Noorden, Vienna). It is a great pleasure here to express to Dozent Eppinger my sincerest thanks for his many helpful suggestions, and aid in carrying out the details of this study.

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THE INFLUENCE OF THE HIGH-CALORY DIET ON THE RESPIRATORY EXCHANGES IN TYPHOID FEVER *

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The influence of a diet rich in carbohydrate on the protein metabolism in typhoid fever[1] and the extent of its absorption[2] have already been studied in detail. The striking clinical effect of the diet in a large number of cases has also been reported.[3] It has seemed desirable, therefore, to investigate the fate of the large amount of food administered and its effect on the total metabolism. During the years 1911 and 1912 it was possible to make 134 determinations of the respiratory exchanges of patients taking the high-calory diet, and the results have been embodied in the following report. Some of the experiments were reported briefly on a former occasion.[4]

Numerous investigations have been made by other observers, but practically all their patients were examined in the fasting state, that is, from six to thirteen hours after the last meal. Moreover, these patients were on diets of low caloric value and were not receiving enough food during the fever to maintain themselves in nitrogen and weight equilibrium. In 1891, Kraus,[5] working with the Zuntz-Geppert apparatus, found that in fever the respiratory quotient depended on the kind of food previously eaten and on the state of nutrition of the body. Following this a number of articles were published in which many of the cited respiratory quotients were so low that they could be explained only on the assumption of a profound change in the laws of metabolism occurring in fever. Chief among those who

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* From the Department of Applied Pharmacology of Cornell University Medical College and the Second Medical Division of Bellevue Hospital.

[1] Shaffer and Coleman: Protein Metabolism in Typhoid Fever. THE ARCHIVES INT. MED., 1909, iv, 538.

[2] Du Bois: The Absorption of Food in Typhoid Fever, THE ARCHIVES INT. MED., 1912, x, 177.

[3] Coleman: The High Calory Diet in Typhoid Fever, a Study of 111 Cases, Am. Jour. Med. Sc., 1912, cxliii, 77; Weight Curves in Typhoid Fever, ibid., 1912, cxliv, 659.

[4] Coleman: Diet and Metabolism in Fever, Tr. Fifteenth International Cong. on Hyg. and Demog., Washington, 1912, ii, 602.

[5] Kraus F.: Ueber den respiratorischen Gasaustausch im Fieber, Ztschr. f. klin. Med., 1891, xviii, 160.

found these low quotients were Robin and Binet,[6] Rolly and Hörnig,[7] Rolly and Meltzer,[8] and E. Grafe.[9] Subsequently, Rolly[10] and Grafe,[11] questioning the correctness of their results, reinvestigated the subject and concluded that the types of apparatus first used were not suitable for the study of the respiratory metabolism in fever. In their later work Rolly and Grafe obtained no quotients below the normal limits, thus confirming the observations of Kraus made many years before. In the meantime Svenson[12] had carefully investigated the metabolic processes of convalescence and had found that the oxygen consumption fell below normal in the first week, rising again above normal during the second week. He found also that a heavy meal increased the metabolism from 30 to 65 per cent., and that during convalescence from typhoid a given amount of work was performed with a greater expenditure of energy than in health. Löning[13] took up the subject of the influence of food on metabolism in fever, and his work indicated that sugar was very rapidly oxidized, the respiratory quotient dropping to its original level within three or four hours after its administration. Glucose had no marked effect in raising the oxygen consumption.

Figure 1 shows graphically all the work done on typhoid patients by Kraus[5] and Svenson[12] and all the later work of Grafe[11] and Rolly.[10] The work of these investigators was selected because it dealt with patients on low diets examined from six to fourteen hours after their last meal; in other words, the basal or *nüchtern* metabolism was determined. This work was selected also because it contained very few quotients below 0.7 and therefore seemed to be technically correct. We have arbitrarily arranged the observations under the headings of "Continued Temperature Period," corresponding to the second week, "Early Steep Curve Period," corresponding to the third

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[6] Robin and Binet: Etudes cliniques sur le chimisme respiratoire, Arch. gén. de méd., 1896, i, 641, ii, 385 and 515.

[7] Rolly and Hörnig: Stoffwechseluntersuchungen an Typhuskranken mit besondere Berücksichtigung des Verhaltens des respiratorischen Quotienten, Deutsch. Arch. f. klin. Med., 1908-1909, xcv, 74.

[8] Rolly and Meltzer: Stoffwechseluntersuchungen bei Fieber, etc., Deutsch. Arch. f. klin. Med., 1909, xcvii, 252.

[9] Grafe, E.: Gaswechseluntersuchungen bei fortgeschrittenen Erkrankungen der Lungen und der Zirkulationsorgane, Deutsch. Arch. f. klin. Med., 1909, xcv, 543.

[10] Rolly, G.: Experimentelle Untersuchungen über den Stoffwechsel im Fieber und in der Rekonvalescenz, Deutsch. Arch. f. klin. Med., 1911, ciii, 93.

[11] Grafe, E.: Untersuchungen über den Stoff- und Kraftwechsel im Fieber, Deutsch. Arch. f. klin. Med., 1911, ci, 209.

[12] Svenson, N.: Stoffwechselversuche an Rekonvalescenten. Ztschr. f. klin. Med., 1901, xliii, 86.

[13] Löning, Karl: Ueber den respiratorischen Gaswechsel im Fieber unter dem Einfluss der Nahrungsaufname, Klin. Jahrb., 1908, xix, 183.

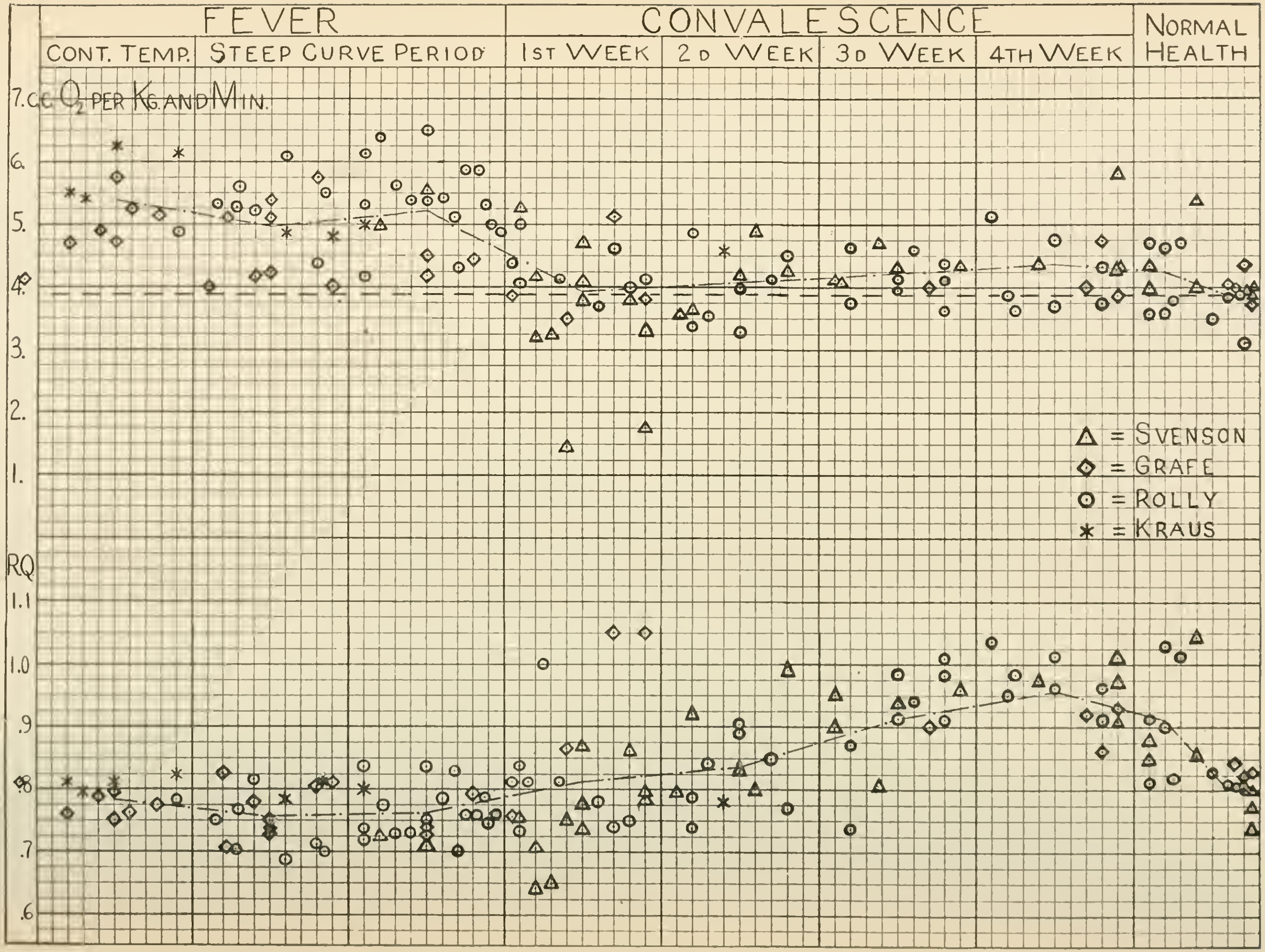

Fig. 1.—The oxygen consumption and respiratory quotients of typhoid patients on low diets examined by Kraus, Svenson, Grafe and Rolly from twelve to fourteen hours after the taking of food.

week, and "Late Steep Curve Period," corresponding to the fourth week of the disease and finally the various weeks of convalescence. In the last column are the results obtained on those patients whose metabolism was investigated several weeks or several months after their recovery.

For purposes of comparison the work on the high-calory cases is summarized in Figure 2, which corresponds to Figure 1 in every particular except that the last column represents the metabolism during relapses.

The high-calory diet used in the cases here reported has been fully described in previous publications.[1, 2, 3]

In brief, it may be said that as soon as typhoid patients are admitted to the hospital they are given food in amounts which are increased as rapidly as their digestions will permit. By the end of a week patients are usually taking a liter of milk, from 300 to 400 c.c. of 20 per cent. cream, from 100 to 200 gm. of lactose, two or three eggs, a couple of slices of toast and from 10 to 30 gm. of butter. This furnishes between two and three thousand calories. One or two thousand more calories are gradually added in the form of larger amounts of the foregoing or in the form of rice, oatmeal, mashed potato, cream of wheat, apple-sauce, custard or ice-cream. It was found that patients could be brought into nitrogen and weight equilibrium even in the active stages of the disease when the temperature was still high.[1] The absorption of the large amounts of food given in this diet was investigated,[2] and it was found that protein and carbohydrates were absorbed as well as in health. The nitrogen of the feces averaged 1.12 gm. a day, which was 7.1 per cent. of the average nitrogen of the food. Carbohydrates were completely absorbed unless more than 300 gm. a day was given, in which case 2 or 3 gm. appeared in the stools. Fats were absorbed in huge amounts, and the loss amounted to only 7.2 per cent. during the first three weeks of the fever and 4.5 per cent. in the later stages of the disease. On an average the patients lost in the feces only 5 per cent. of the calories ingested.

EXPERIMENTAL PROCEDURE

The type of apparatus selected for the investigation was the small "unit" or "universal apparatus" of Benedict.[14] In 1911 the machine resembled that described in Benedict's first publication, except that

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[14] Benedict, F. G.: An Apparatus for Studying the Respiratory Exchange, Am. Jour. Physiol., 1909, xxiv, 345; Ein Universalrespirationsapparat, Deutsch. Arch. f. klin. Med., 1912, cvii, 156.

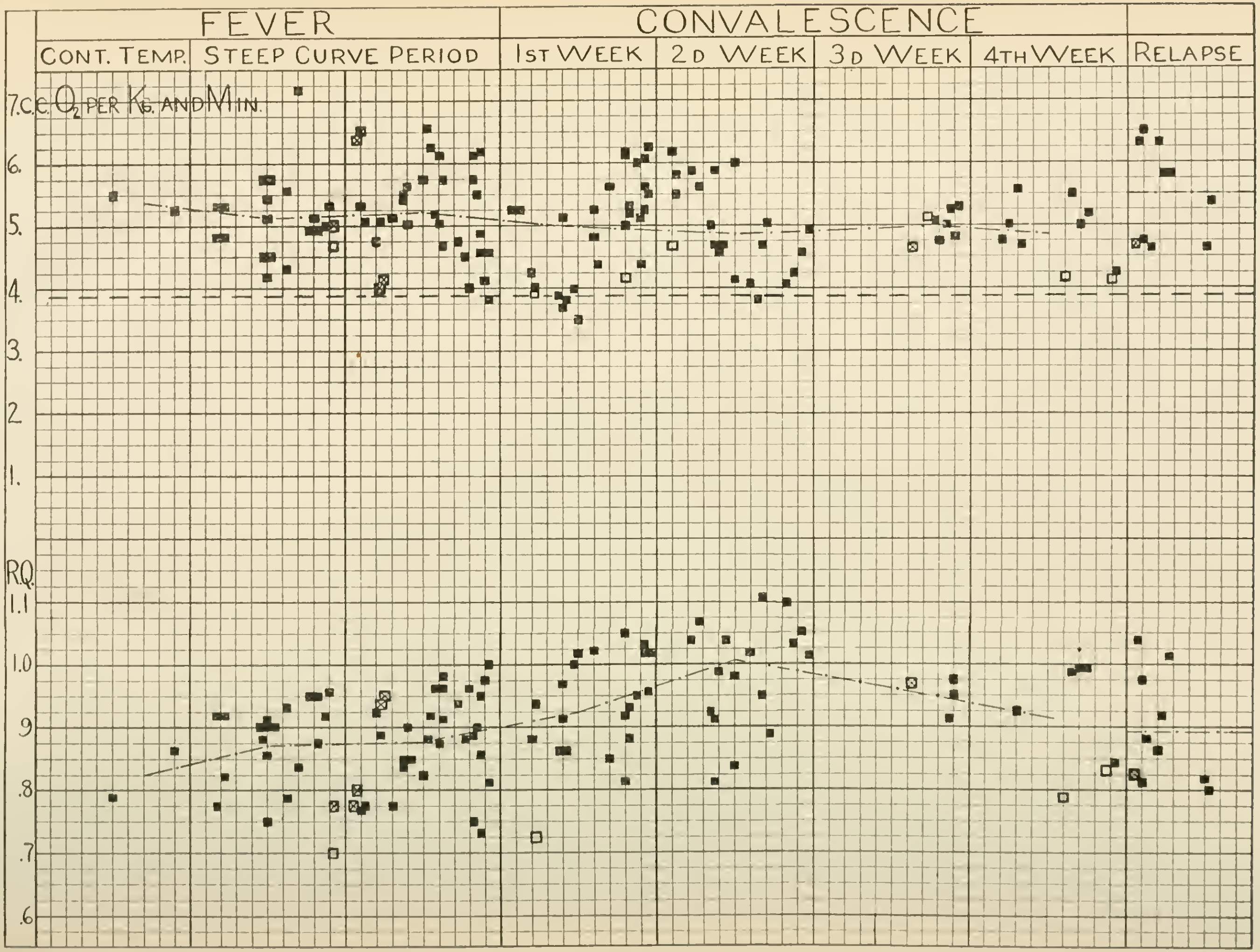

Fig. 2.—The oxygen consumption and respiratory quotients of typhoid patients on high-calory diet. Solid squares indicate observations made shortly after meals; open squares, fasting observations; crossed squares, observations, made from five to seven hours after the last meal or shortly after a very light meal.

Williams bottles[15] were used instead of the Woulfe bottles and Kipp generators, thus reducing the volume of air within the apparatus.

During 1912 a spirometer was substituted for the rubber bag, and the apparatus more closely resembled that described in Benedict's second publication. The old style of air-moistener and the single absorber system were retained but, unfortunately, the work-adder was not attached until the typhoid season was over. The nose-pieces require a special description, as they differed from Benedict's model. A soft rubber tube, reinforced by a spiral of brass wire, was substituted for the glass core used by Benedict, and the finger-cot was tied around this as shown in Figure 3. In order that the direction of the nose-pieces might be changed to fit different noses, a pair of wire spreaders, also shown in Figure 3, was attached. With some of the patients it was difficult to secure an air-tight joint when the finger-cot between the core and the inside of the nose was inflated. The flexibility of the new nose-pieces and the spreaders improved conditions considerably, but it seemed necessary to take the precaution of lathering the joint between nose-piece and nose with soap. This lather was watched during the whole experiment by one or two observers. and leaks were sometimes discovered which would otherwise have passed unnoticed. The use of lather with this apparatus is strongly recommended, since the loss of any quantity of air increases by a corresponding amount the apparent consumption of oxygen.

The experimental procedure is important enough to require a detailed description. The respiration apparatus was wheeled to the patient's bed and the oxygen bomb and the CO_2 absorbing system weighed to within 0.01 gm., all weighings being checked by an assistant. The bottles were then connected with the machine and the whole tested for leaks under a slightly increased pressure while the air was circulating at full speed. This was continued for some time in order to bring the various parts of the machine to a constant temperature. Meanwhile the subject's mouth was sealed with adhesive plaster, and the nose-pieces inserted, blown up and lathered. All this time a valve was turned so that the patient was breathing room air. Next the pressure within the machine was brought almost to zero and the level of the oil manometer carefully recorded. Then while the patient was breathing quietly and regularly, the valve was turned at the end of a normal expiration and he began to breathe the air which was circulating in the machine. During the experiment the pulse and respiration were counted at least three times and the character of respiration recorded. Note was made of each movement of the patient, although none of them did more than move a hand or leg a few inches. As the experiment progressed, oxygen was admitted by means of a reducing valve on the oxygen cylinder, and the rubber bag kept at approximately the same level. At the end of a period, which was almost always fifteen minutes, the valve near the nose-piece was quickly reversed, exactly at the end of a normal expiration. The air was then kept in circulation for two or three minutes in order to remove the last traces of carbon dioxid which might be in the passageways of the machine. Next oxygen was gradually admitted until the oil manometer gave the same reading as at the start of the experiment. Both readings were made about one minute after the circulation of the air was stopped. In 1912, when the spirometer was used, graphic records of the respiration were made in the manner described by Benedict,[16] and the oxygen was admitted until the original level of the spirometer was reached. The carbon dioxid absorbers and oxygen bomb were immediately weighed and the calculations made as follows:

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[15] Williams, H. B.: Animal Calorimetry, First Paper, A Small Respiration Calorimeter, Jour. Biol. Chem., 1912, xii, 317.

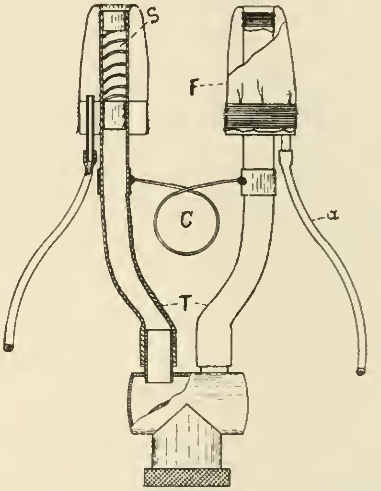

Fig. 3.—Diagram of modified nose-pieces: *S,* flexible spiral spring; *F,* finger-cot; *C,* spreaders for adjustment of nose-pieces; *T,* soft rubber tubes; *A,* small rubber tube for inflating finger-cots.

$$\frac{CO_2 \text{ gm.} \times 0.509}{O_2 \text{ gm.} \times 1.004 \times 0.669} = \frac{CO_2L}{O_2L} = \text{Respiratory Quotient.}$$

The correction of adding 0.4 per cent. to the oxygen was made, since the Linde Air Product Company's oxygen was found on analysis to contain the same percentage of impurity that Benedict[16] found.[17]

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[16] Benedict, F. G: Universalrespirationsapparat, Deutsch. Arch. f. klin. Med., 1912, cvii, 156.

[17] Shortly before this article was ready for publication, our attention was called to the fact that G. W. Morey of the United States Bureau of Standards had discovered that 98 per cent. of the impurity in the Linde Air Product Company's oxygen was argon and not nitrogen as had been previously supposed.

In all cases in which the urinary nitrogen was determined, the method described by Loewy[18] was used. This showed that in every case except one (Frank W.), protein furnished very close to 15 per cent. of the total calories. It was therefore possible to use the tables of Magnus-Levy[19] in those cases in which it was impossible to obtain the urinary nitrogen. These two sets of tables gave the caloric value of the oxygen for each experiment and made it easy to calculate the calories produced by the patient during each fifteen-minute period. That this method of indirect calorimetry corresponds very closely to the direct calorimetry has been shown by the animal experiments of Lusk and by unpublished experiments on human subjects.

The accuracy and general utility of the Benedict respiration apparatus have been proved by Benedict and his coworkers[20] in many comparisons with the Atwater-Rosa-Benedict respiration calorimeters. It must be remembered that all experimental difficulties are greatly increased in working with typhoid patients. It is almost impossible to keep a patient on a rigidly specified diet, and in the case of patients with high fever it is not justifiable to make more than two or three experiments a day. As a rule, the patients are somewhat nervous the first time they use the apparatus, and it has seemed advisable to leave unpublished the first observations on all except two phlegmatic individuals who showed absolutely no apprehension. Patients soon become accustomed to the procedure, however, and rather enjoy the break in the day's monotony, since they suffer no discomfort.

It was difficult to begin the experiments on schedule time since leaks would often be discovered at the nose just before the start. After the start a rising and falling bubble in the lather around the nose-pieces sometimes made it necessary to interrupt the period for a few minutes and readjust the apparatus. If the bubble broke or

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(The Occurrence of Argon in Commercial Oxygen made from Liquid Air, Jour. Am. Chem. Soc., 1912, xxxiv, 491). On account of the difference in the atomic weights of these gases, the correction should have been —0.6 per cent. instead of +0.4 per cent. This means that all the oxygen figures are about 1 per cent. too high and that the respiratory quotients are a trifle too low. The error is so small that it has seemed unnecessary to recalculate all the results given in this article and in the publications from Benedict's laboratory, which have been used as controls.

[18] Loewy: Oppenheimer's Handbuch. der Biochemie, 1911, iv.[1] 277.

[19] Magnus-Levy: Von Noorden's Handbuch der Pathologie des Stoffwechsels, Ed. 2, 1906, i, 207.

[20] Benedict and Joslin: Metabolism in Diabetes Mellitus. Carnegie Inst. of Washington, 1910, Bull. 136; A Study of Metabolism in Severe Diabetes, ibid., 1912, Bull. 176; Benedict, Emmes and Riche: The Influence of the Preceding Diet on the Respiratory Quotient After Active Digestion has Ceased, Am. Jour. Physiol., 1911, xxvii, 383.

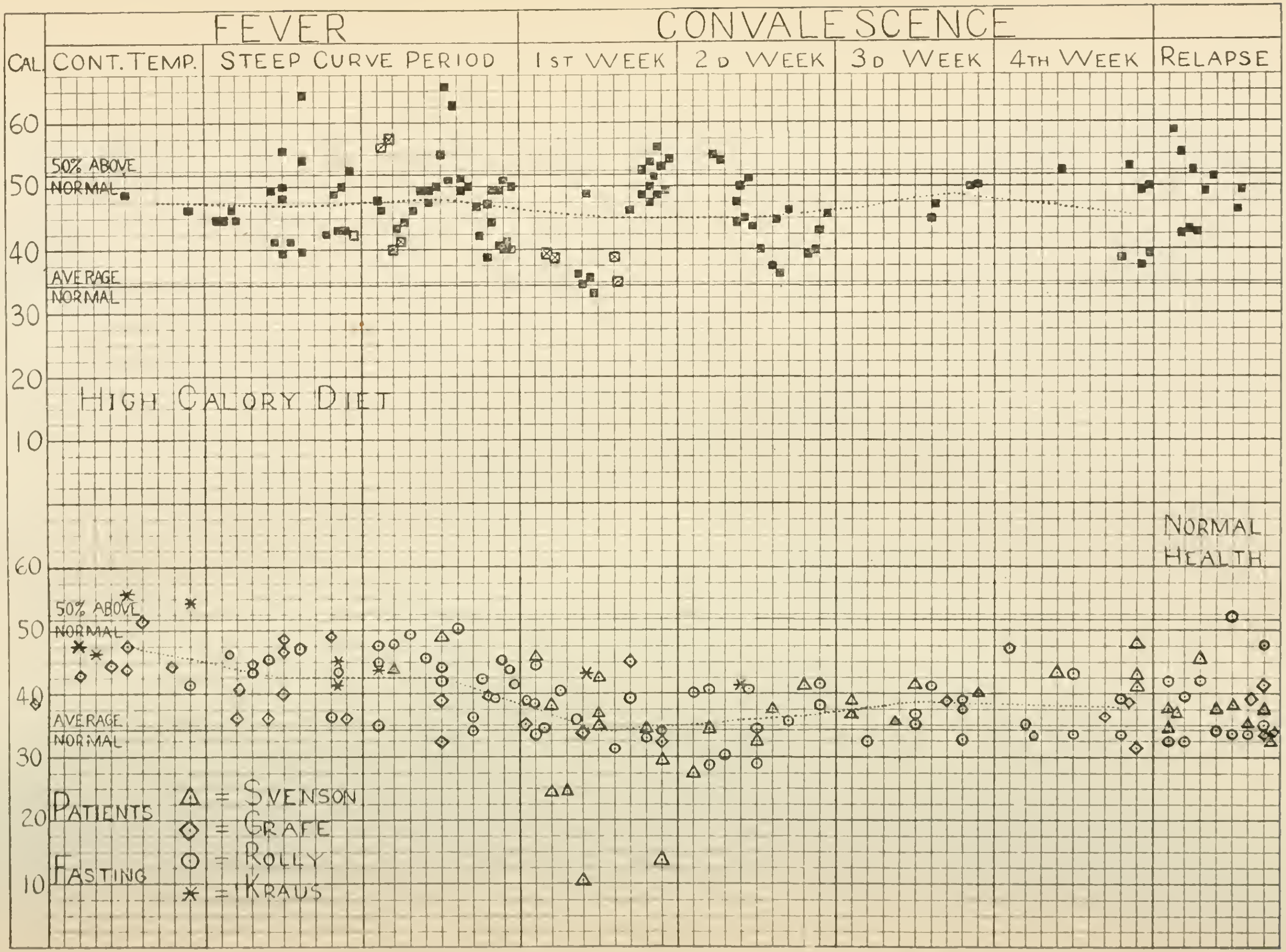

Fig. 4.—Results expressed in terms of calories per square meters of body-surface per hour. Comparison of high-calory cases examined shortly after meals and the fasting cases of Kraus, Svenson, Grafe and Rolly.

if there were any other leak, the experiment was discarded. The chief technical difficulty, however, lay in the tendency of some of the patients to increase the frequency or depth of their respirations shortly after the period was started. A few of the febrile patients persisted in this type of breathing, and it was necessary to give them up as experimental subjects. Two patients, Louis M. and Frank W., who had been breathing normally during the fever, developed this tendency early in convalescence, but their data were retained and grouped in a special table. The cause of the trouble is not clear; it might be due to nervousness on the part of the patient or to some unknown mechanical effect of the apparatus.

An increasing rate of ventilation (*Auspumpung*) has a very serious effect on the carbon dioxid determination, especially if the experimental period be a short one. Between the tissues whose carbon dioxid production we wish to estimate and the apparatus, there are from 3 to 5 liters of blood and 2.5 liters of residual air. While the carbon dioxid content of these does not change if the rate of ventilation be uniform, there is an immediate decrease of the carbon dioxid of the alveolar air when the alveolar ventilation is increased. This, in turn, removes some of the carbon dioxid from the blood, in which a large part is held in loose combination. Consequently, there is a "pumping out" of carbon dioxid from blood and residual air. There are no similar depots for oxygen in the body as has been shown by Katzenstein,[21] Zuntz and Geppert[22] and Loewy,[23] and the increased ventilation causes practically no change in the oxygen consumption. The percentage of oxygen in alveolar air may be slightly increased, as may the relatively small amount of oxygen held in solution in the blood-plasma. There may also be a slight increase in oxygen consumption because of the extra work of the muscles of respiration. It is evident that if stored carbon dioxid is exhaled in addition to the normally produced carbon dioxid and the oxygen remains constant, the respiratory quotient will be too high.

The effects of such increased ventilation (*Auspumpung*) are shown in a series of experiments on normal persons (Table 1). The first subject was examined during a period with natural breathing which gave his true oxygen and carbon dioxid. One hour later another fifteen-minute experiment was made in which the breathing was as deep and as rapid as possible. This increased the oxygen consumption 21 per cent., since the muscular work was severe. A second subject breathed deeply but not excessively during the first ten of the fifteen minutes and raised the respiratory quotient from 0.75 to 1.05, but increased the oxygen intake by only 5 per cent. The next subject was Louis M., one of the typhoid patients who had developed the tendency to *Auspumpung* during convalescence. Five months after leaving the hospital cured he returned to serve as a normal control. His fasting metabolism and respiratory quotient were determined in a respiration calorimeter in which *Auspumpung* was impossible, and two days later, under exactly similar conditions, two observations were made with the small Benedict apparatus. Curiously enough, the abnormal type of breathing had persisted and it was possible to compare his unintentional *Auspumpung* with a faultless calorimeter experiment. The increase in oxygen consumption amounted to 4 and 5 per cent. in the two periods with the small apparatus.

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[21] Katzenstein: Ueber die Einwirkung der Muskelthätigkeit auf den Stoffverbrauch des Menschen, Arch. f. d. ges. Physiol. (Pflüger's), 1891, xlix, 330.

[22] Zuntz and Geppert: Ueber die Regulation der Athmung, Arch. f. d. ges. Physiol. (Pflüger's), xlii, 189.

[23] Loewy: Die Wirkung ermüdende Muskelarbeit auf dem respiratorischen Stoffwechsel, Arch. f. d. ges. Physiol (Pflüger's), 1891, xlix, 405; Der respiratorische und der Gesamtumsatz, Oppenheimer's Handbuch der Biochemie, 1911, iv, p. 154, 161.

TABLE 1.—Experiments in "Auspumpung" on Normal Persons from Fourteen to Seventeen Hours After Last Meal

	Periods 15 Min. Start of Exper. a. m.	Per Kg. and Min.		R. Q.	Increase in O_2 from *Auspumpung*, Pct.
		CO_2 c.c.	O_2 c.c.		
D. B., wt. 75.3 kg.:					
Oct. 31, normal breathing.....	10:35	2.69	3.48	0.77	
Breathing as deep and as fast as possible	11:35	4.62	4.21	1.10	21
J. R., wt. 66 kg. (approx.):					
Nov. 25, deep breathing first 10 minutes	10:37	4.43	4.24	1.05	5
Normal breathing	11:35	3.04	4.06	.75	
Louis M., wt. 51.7 kg.:					
March 26, normal breathing, 6 hours in calorimeter	10:10	3.36	4.34	.77	
March 29, unintentional *Auspumpung*	11:11	4.97	4.52	1.10	4
March 29, unintentional *Auspumpung*	11:55	5.03	4.57	1.10	5

The *Auspumpung* of the few typhoid patients who exhibited this phenomenon was of the type which, in the controls, raised the oxygen figures from 4 to 5 per cent. and never approached the maximal effort of the first normal subject. Since the carbon dioxid and respiratory quotient of all *Auspumpung* observations are too high, it has been necessary to group the experiments in which this phenomenon probably occurred in a special table. The quotients have been discarded, but the oxygen figures are so slightly changed that it seemed advisable to include them in the graphic chart (Fig. 2) and in the table of averages.

While the present work was in progress, Rolly and Rosiewitz[24] published an article on typhoid describing a modification of the Benedict apparatus changed to meet certain objections which they raised in regard to the original machine. These objections have been fully answered by Benedict,[16] but the best answer is to be found in the close agreement between the small apparatus and the large respiration calorimeters of the Nutrition Laboratory in Boston and the Russell Sage Institute of Pathology in New York. The latter apparatus has not yet been described.*

Kraus[5] and Svenson[12] used with apparent success the Zuntz-Geppert apparatus. Grafe,[11] in his recent work on typhoid, used a respiration cabinet of the Jacquet type in which he kept his patients from three to six hours. In this manner he avoided all possibility of *Auspumpung.*

CALCULATION OF FOOD

In the season of 1911 it was impossible to weigh and analyze each kind of food as was done in 1912. The patients, however, were under the personal charge of the head nurse who had assisted in metabolism experiments for three years. Milk and cream, the chief articles of the diet, were carefully measured and were analyzed from time to time. Lactose was also measured in a specially graduated cup. The other foods were given in standard portions which were checked up at frequent intervals. Calculations were based on analyses made in this clinic and on the tables of Atwater and Bryant.[25] In reckoning the calories of the food the factors of Rubner, 9.3 for fat and 4.1 for protein and carbohydrate, were used.

In 1912, it was possible to secure the services of three nurses for the typhoid cases alone. In this year cereals which had been analyzed were weighed raw and each portion separately cooked. Bread, eggs, ice-cream and a few other foods were analyzed and weighed out in the moist condition, and milk and cream were measured in graduates.

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* A preliminary report will appear shortly in the Jour. Am. Med. Assn.

24. Rolly and Rosiewitz: Deutsch. Arch. f. klin. Med., 1911, ciii, 58; Rolly: Bemerkungen zu dem Aufsatz von F. G. Benedict, Ein Universalrespirationsapparat, ibid., 1912, cvii, 593.

25. Atwater and Bryant: The Chemical Composition of American Food Materials, U. S. Dept. of Agric., Bull. 28, Revised Edition, 1906.

NORMAL CONTROLS

Twelve fasting experiments on three normal controls were made with the Benedict apparatus and the results grouped in Table 2. All the controls were men thoroughly familiar with the apparatus. D. B. was a physician aged 30, 5 feet 9¾ inches in height. F. C. G. was a chemist aged 29, 5 feet 8½ inches in height. Louis M., a barber, was the typhoid patient mentioned above, who returned five months after his attack in order to serve as a control. His type of respiration on the machine remained abnormal and it was necessary to use the respiratory quotient of a calorimeter experiment to obtain the caloric value of the oxygen.

The heat production of these controls has been calculated in two ways for purposes of comparison. In the first column use has been made of the urinary nitrogen, and the tables of Loewy[18] have furnished the caloric value of the oxygen according to the non-protein respiratory quotient. In the second column the simpler and somewhat less exact method of reckoning that the protein furnished 15 per cent. of the calories as given by Magnus-Levy[19] has been used. It will be seen that the two columns agree very closely.

The same controls served as subjects for experiments with the respiration calorimeter of the Russell Sage Institute of Pathology. The figures for the direct calorimetry and for the indirect calorimetry using the tables of Loewy, are given in Table 3. Unfortunately the calorimeter leaked a little air in both experiments on F. C. G., and his oxygen consumption and indirect calorimetry were not determined. Table 4 gives the averages of all the calorimetric determinations by the four different methods, and shows a striking agreement between all four. Benedict[26] has repeatedly demonstrated the fact that figures obtained with his small apparatus correspond closely with those obtained with the calorimeter.

Table 5 gives the average oxygen consumption and heat production of forty-eight normal controls whose metabolism was investigated in short periods. Twenty-three of these are taken from Benedict, Bulletin 176, Table 131; four from Benedict, Bulletin 136, Table 166; nineteen from Loewy and Magnus-Levy, quoted by Benedict in Bulletin 136, Table 161. Our two normal controls, D. B. and F.C.G., have been included in the list. The tables as published by Benedict do not give the indirect calorimetry, and this has been calculated. The average respiratory quotient of Benedict's cases in Bulletin 176 is 0.834, of Loewy's and Magnus-Levy's cases, 0.781, of our cases, 0.779.

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26. Benedict and Joslin: Metabolism in Diabetes Mellitus, Carnegie Inst. of Washington, 1910, Bull. 136; A Study of Metabolism in Severe Diabetes, ibid., 1912, Bull. 176.

TABLE 2.—Basal Metabolism of Normal Controls Determined with Benedict Respiration Apparatus from Fourteen to Eighteen Hours After Last Meal

Subject, Date and Weight (kg.)	Start of Experiment	Average Pulse-Rate	Average Respiration Rate	R. Q.	Per Kg. and Min.			Cal. Per Kg. and 24 Hrs. Indirect Calorimetry	
					CO₂ c.c.	O₂ c.c.	N. of Urine, Mg.	Reckoned from Tables of Loewy, Using Urinary N	Reckoned from Tables of Magnus-Levy (15% Cal. from Protein)
D. B., Oct. 31, 1912, 75.3	9:30 a. m.	62	12	0.71	2.66	3.73	0.132	25.0	25.1
	10:35 a. m.	61	13	.77	2.69	3.48	0.132	23.5	23.7
D. B., Dec. 13, '12, 75.3	11:34 a. m.	60	12	.73	2.62	3.59	0.116	25.0	24.2
	12:14 p. m.	60	12	.74	2.93	3.97	0.116	27.6	26.8
	12:51 p. m.	62	12	.79	2.79	3.56	0.116	24.9	24.4
	1:29 p. m.	61	12	....	2.77	3.68	0.116	25.6	25.0
F. C. G., Dec. 16, '12, 56.5	11:55 a. m.	76	12	.81	3.26	4.05	0.138	27.7	27.9
	12:16 p. m.	74	13	.86	3.29	3.84	0.138	26.6	26.8
F. C. G., Dec. 17, '12, 56.5	11:32 a. m.	75	15	.78	3.24	4.15	0.107	28.2	28.3
	11:52 a. m.	71	14	.82	3.13	3.82	0.107	26.2	26.4
	12:13 p. m.	70	13	.78	2.99	3.86	0.107	26.2	26.4
Louis M., March 29, '13, 53.35. *Auspumpung*	11:11 a. m.	75	15	1.10*	4.97*	4.52	0.199	....	30.8*
	11:55 a. m.	85	17	1.10*	5.03*	4.57	0.199	....	31.1*

* Caloric value of oxygen for respiratory quotient of 0.77 as determined two days previously in respiration calorimeter used instead of the caloric values for these quotients, which are too high on account of *Auspumpung.*

TABLE 3.—BASAL METABOLISM OF NORMAL CONTROLS DETERMINED WITH RESPIRATION CALORIMETER FROM FOURTEEN TO EIGHTEEN HOURS AFTER LAST MEAL

Subject, Date and Weight	Time of Experiment	R. Q.	Per Kg. and Min.			Calories per 24 Hours per Kilogram		Cal. per Hour per Square Meter Body-Surface
			CO_2 c.c.	O_2 c.c.	N. of Urine, Mg.	Indirect Calorimetry	Direct Calorimetry	
D. B., March 13, '13, 73.52	9-35-11:35	0.82	3.03	4.11	0.126	25.5	24.5	36.29
D. B., May 17, 75.51	9:30-12:30	.83	2.84	3.41	0.116	23.4	22.7	34.60
F. C. G., March 17, 56.50	9:02-12:02	....	3.38	....	0.145	....	24.3	32.80
F. C. G., April 22, 54.87	9:45- 2:45	....	3.37	....	0.095	....	26.6	35.17
Louis M., March 26, 51.7	10:10- 4:10	.77	3.36	4.34	0.168	29.3	30.0	37.74
Average normal*		....	....	....	....	....	....	34.20

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* Average of 12 normal controls in the bed calorimeters of the Russell Sage Institute of Pathology, unpublished, and the Nutrition Laboratory in Boston, 3.44.

As a mean of these the quotient of 0.8 has been selected to give the caloric value of the oxygen. According to the table of Magnus-Levy, with a quotient of 0.8, 1 liter of oxygen represents 4.77 calories. This factor has been used for all the controls.

TABLE 4.—Normal Controls: Comparison of Atwater-Rosa-Benedict Respiration Calorimeter and Benedict Respiration Apparatus; Averages of all Experiments from Fourteen to Sixteen Hours After Last Meal

Subject	C.C. O_2 Per Kg. and Min.		Calories Per Kg. and Twenty-Four Hours			
			Direct Calorimetry Bed Calorimeter	Indirect Calorimetry		
				Calorimeter	Benedict Apparatus	
	Calorim.	Benedict.			Reckoned from Urinary Nitrogen	Reckoned from Tables of Magnus-Levy
D. B.	3.76	3.67	23.6	24.3	25.3	24.9
F. C. G....	....	3.95	25.4	....	27.0	27.2
Louis M...	4.34	4.54	30.0	27.3	....	31.0

TABLE 5.—Normal Oxygen Consumption and Heat Production: Averages of Forty-Eight Normal Controls from Various Sources Grouped According to Body-Weight

Weight, Kg.	Number of Subjects	O_2 C.C. Per Kg. and Min.			Av. Calories	
		Min.	Max.	Average	Per Kg. per 24 Hours	Per Sq. M. Body Surface per Hour
40-50	6	3.68	4.57	4.06	27.9	33.6
50-60	17	3.32	4.41	3.86	26.5	34.1
60-70	15	3.47	5.50	3.71	25.5	34.5
70-80	4	3.15	3.73	3.48	23.9	34.1
80-90	6	2.76	3.60	3.16	21.7	32.3

The subjects have been grouped according to body-weight, and it will be seen that the calories produced per kilogram, as is well known, decrease as the weight increases. On the other hand, there is a striking uniformity in the column showing calories per square meter of body-surface, as Rubner demonstrated many years ago. The surface area was calculated from Meeh's formula, using the body-weights of 45, 55, 65, etc., and using the average heat production of individuals of the same weight group. The subjects between 80 and 90 kg. show an unexpectedly low average metabolism, and it would be interesting to see if a larger number of controls of this weight would give the same results. Seven normal controls have been studied in the respiration calorimeter of the Russell Sage Institute of Pathology, and they have been averaged with five normal controls from figures published by Benedict.[27] The average heat production per square meter of body-surface per hour is 34.2 calories with a variation 10 per cent. above or 10 per cent. below this point. The results will shortly be published in detail.

It is more accurate to express the results of respiration experiments in calories per square meter of body-surface, since it then becomes possible to compare individuals of different weights. In the tables of respiration experiments, both calculations are given, but the final comparisons are made according to body-surface. For the clinicians' purposes calculations based on a twenty-four-hour period are best, but from the point of view of the experimenter the one-hour period is preferable since it is much closer to the actual experimental time of all types of apparatus, except a few of the large respiration cabinets. The habit of comparing results in cubic centimeters of carbon dioxid and oxygen per kilogram and minute has nothing to recommend it except the fact that previous observers have used the method and have made it necessary to follow their example for purposes of comparison.

HISTORIES OF PATIENTS

Joseph T., weight 58.4 kg., aged 35, of medium build. Typhoid fever of severe type. Admitted Oct. 13, 1911, the twenty-fifth day of disease. Discharged November 21. Fever lasted forty-one days, no relapse. Vomited once; otherwise no gastric or intestinal disturbance. Not restless. See table for clinical data.

John H., colored, weight 54.3 kg., aged 27, of medium frame. Typhoid fever, moderately severe type. Admitted Oct. 23, 1912, on sixteenth day of disease. Discharged December 28. Fever lasted thirty-three days. Took food fairly well. Not delirious or restless. See table for clinical data.

Frank W., weight 54.5 kg., aged 27, short and stout. Typhoid fever of severe type. Admitted Nov. 23, 1911, tenth day of disease. Discharged February 8. Original fever lasted thirty-two days. A relapse of moderate severity began on the forty-seventh day and lasted to the sixty-first day. Glycosuria appeared on the fifteenth day, lasting till the twenty-first day. No history of diabetes in patient's family or in himself. Severe sore throat, January 7. Vomited at start; could not take milk with lactose. Soon began to take food better, but barely reached 3,000 calories a day until temperature was falling to normal. No diarrhea or tympanites. Not restless. See table for clinical data.

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27. Benedict and Joslin: Metabolism in Diabetes Mellitus, Carnegie Inst. of Washington, 1910, Bull. 136.

Davide G., weight 56.1 kg., aged 27, short and muscular. Typhoid fever of severe type. Admitted Oct. 27, 1911, twenty-first day of disease. Discharged December 5. Fever lasted forty days, no relapse. Ugly tempered. Took food poorly in early stage. Appetite began to improve November 1. No diarrhea. Not restless.

Twenty-seventh day of disease, T. 101.2-104; 63 food calories per kilogram. Twenty-eighth and twenty-ninth days, T. 99.8-103.2; 72-73 calories per kilogram. Thirtieth day, T. 99.0-102; 71 calories per kilogram. Thirty-first day, T. 99.2-101.4; 64 calories per kilogram. Thirty-second day, T. 99.2-100.4; 76 calories per kilogram. Thirty-third day, T. 99.5-101.2; 80-89 calories per kilogram. Thirty-seventh day, T. 98.6-100.2; 78 calories per kilogram.

John C., weight 62.3 kg., build, medium, slight. Typhoid of severe type. Admitted to medical service Aug. 22, 1911, probably twenty-eighth day of disease (transferred from Psychopathic Pavilion). Discharged to Psychopathic Pavilion October 5. Original fever lasted about fifty days. No relapse. Looked sick. Diarrhea on admission, none later. No tympanites. Took food well at the start — not so well later. Gained 1 pound a day from thirty-first to thirty-eighth days. Slept greater part of time. Mental condition recorded as dull and stupid.

Thirty-fifth day of disease, T. 100.0-102; 54 food calories per kilogram. From the thirty-sixth to the thirty-eighth day, T. 99.2-104.7; from 63 to 71 calories per kilogram. Thirty-ninth day, T. 100.2-103.2; 53 calories per kilogram.

Abe B., weight 74.8 kg., aged 20, short and stout. Typhoid fever of mild type. Admitted to genito-urinary service for gonorrheal epididymitis, Oct. 29, 1911, probably the twenty-first day of the typhoid. Transferred to medical service on the thirty-second day. Discharged December 19. Long, low fever uninfluenced by epididymitis, temperature rising to 100.6 the day before discharge on the seventy-second day. Nausea only twice. No diarrhea. Not restless.

Thirty-seventh day, T. 99.0-101.0; 55 food calories per kilogram. Thirty-eighth day, T. 99.4-100.3; 46 calories per kilogram. Thirty-ninth day, T. 98.4-101.2; 64 calories per kilogram. From the twentieth to the forty-second days, T. 99.1-101.6; 57-64 calories per kilogram. Forty-third day, T. 98.2-100.7; 48 calories per kilogram.

Anton V., weight 62.3 kg., aged 32, tall and spare. Typhoid of severe type. Admitted Aug. 13, 1911, the twenty-eighth day of disease. Discharged October 11. Original fever lasted thirty-four days. Patient was afebrile for six days and then had relapse of eleven days' duration. Complicated by phlebitis September 4. Took food well. No gastric or intestinal disturbance. Not restless.

Thirty-third day of disease. T. 99.2-100.6; 40 food calories per kilogram. Convalescence, from the first to the third day, from 51 to 58 calories per kilogram. Relapse, first to sixth days, T. 97-104.2; from 56 to 61 calories per kilogram. Seventh day, T. 98.8-101.8; 47 calories per kilogram. Eighth day relapse to ninth day convalescence, from 71 to 110 calories per kilogram. Tenth day, 108 calories per kilogram. Eleventh to twentieth days, from 86 to 117 calories per kilogram. Twenty-first day food not measured; approximately 100 calories per kilogram.

Frank R., weight 61.6 kg., aged 18, of medium build. Typhoid fever of severe type. Admitted Oct. 2, 1911, twenty-first day of disease. Discharged November 22. Original fever lasted twenty-eight days. No relapse. No gastric or intestinal disturbance. Not restless.

Charles S., weight 57.7 kg., aged 21, of medium height. Typhoid fever of moderate severity. Admitted Aug. 5, 1911, the third day of disease. Discharged convalescent, October 13. Original fever lasted twenty-one days. Relapse on eighteenth day, fever continuing to fifty-first day. Last experiment on twen-

TABLE 6.—CLINICAL DATA, FRANK W.

Day of Disease	Temperature		Food					Urine, N.	Feces, N.	N, Balance	Urine, Glucose*	Urine, Kreatinin	Body - Wt., kg.
	Min.	Max.	Total Cal.	Cal. per Kg.	Fat, Gm.	Carb., Gm.	N., Gm.						
11	103.0	104.4	2,140	39	105	160	14.0	11.0	1.4†	+1.6	0.0	....	
12	102.0	104.0	2,850	52	139	290	14.1	14.1	1.4†	—1.4	....	1.01	54.5
13	102.0	105.4	2,100	39	110	192	11.2	16.3	1.1†	—6.2	±	1.4	
14	103.2	104.4	1,910	35	67	276	5.9	18.5	1.4	—14.0	±	1.67	
15	102.4	104.0	3,380	62	170	334	16.9	15.2	1.4	+0.3	7.7	1.28	54.5
(Early Steep-Curve Period)													
16	102.2	103.0	2,510	46	125	267	9.7	14.3	1.4	—6.0	8.0	1.27	53.6
17	101.2	103.0	3,700	68	199	343	17.4	18.9	1.4	—2.9	15.5	1.35	
18	101.0	103.0	3,860	71	192	397	17.2	19.9	1.4	—4.1	31.6	1.59	
19	100.4	103.0	3,700	69	190	373	15.4	17.9	1.4	—3.9	79.6	1.29	
20	99.4	102.4	2,130	40	202	173	17.0	13.8	1.0	+2.2	11.0	.99	
21	99.2	103.6	2,670	50	183	128	17.1	19.9	1.0	—3.8	8.5	1.17	
22	100.0	103.0	1,860	35	139	65	11.4	12.9	1.0	—2.5	0.0	1.05	
(Late Steep-Curve Period)													52.7
23	98.0	101.0	2,860	54	200	131	17.9	18.8	.8	—1.7	0.0	1.11	
24	98.4	100.8	3,980	76	282	202	20.5	16.5	.8	+3.2	0.0	....	52.5
25	98.8	101.8	4,100	78	288	218	20.6	14.3	.8	+5.5	1.6	1.12	
26	98.6	101.4	3,750	71	265	195	19.0	11.9	.8	+6.3	1.6	....	
27	98.4	100.4	3,740	71	257	203	20.1	12.3	.8	+7.0	1.8	1.06	53.9
28	98.3	101.3	4,100	76	288	215	21.1	17.4	.8	+2.9	1.6	1.29	
29	98.4	100.4	3,910	73	266	221	20.6	13.9	2.1†	+4.1	1.2	1.01	53.9
30	98.2	100.2	3,670	68	249	206	19.9	....	.....	....	....	.6	
31	98.6	100.0	4,000	74	275	237	18.7	12.0	1.9†	+4.8	1.1	.9	54.3
(Convalescence)												1.21	
1	98.0	99.2	3,990	73	275	224	20.1	14.3	2.0†	+3.8	1.6	.....	
2	98.2	99.8	3,860	71	247	276	17.2	12.2	1.7†	+3.3	0.0	.89	
3	98.0	98.8	3,930	72	248	294	16.0	9.1	1.6†	+5.3	1.0	.88	
4	98.0	99.2	4,590	83	306	301	20.3	12.3	2.0†	+6.0	1.5	1.34	55.1
5	98.0	99.2	2,300	42	151	151	10.5	9.8	1.0†	—0.3	0.0	....	
6	97.7	99.1	3,700	68	258	208	17.7	10.5	1.8†	+5.5	1.4	1.05	54.8
7	98.2	98.8	2,710	49	180	160	14.5	8.7	1.4†	+4.3	0.0	.94	
8	98.0	99.4	3,840	71	241	274	18.3	10.0	1.8†	+6.5	0.0	1.11	54.1

* In this column, ± means trace.
† Feces nitrogen assumed to be 10 per cent. food nitrogen, in cases in which feces were not analyzed.

tieth day. Severe recurrent epistaxis from the twenty-sixth to the thirty-eighth day caused a suspension of the observations. Took food well. No diarrhea or tympanites. Not restless.

Tenth day of disease, T. 99.5-100.8; 75 food calories per kilogram. Eleventh day, T. 99.5-101; 67 calories per kilogram. Twelfth day, T. 99.5-101.4; 49 calories per kilogram. Thirteenth and fourteenth days, T. 98.4-100.0; from 61 to 76 calories per kilogram. Fifteenth day, T. 99.8-102.7; 77 calories per kilogram. Sixteenth to eighteenth days, T. 96.8-99.8; from 75 to 78 calories per kilogram. One day relapse, T. 100.8-102.8; 54 calories per kilogram. Second day, T. 102-103; 67 calories per kilogram.

William M., weight 67.3 kg., aged 32, of moderate height, rather stout. Typhoid moderately severe. Admitted October 16, 1911, the twentieth day of disease. Discharged November 9. Complication serofibrinous pleurisy. Original fever lasted thirty-eight days. No relapse. Good appetite. No gastric disturbance or diarrhea. Not restless.

Twenty-third day of disease, T. 102-103; 89 food calories per kilogram. Twenty-fourth day, T. 100.5-103; 92 calories per kilogram. Twenty-fifth day, T. 100.2-103; 83 calories per kilogram. Twenty-sixth to thirtieth days, T. 99.2-102; from 68 to 87 calories per kilogram. Thirty-first day, T. 99-101; 76 calories per kilogram. Thirty-second day, T. 98.8-101; 66 calories per kilogram.

Archie C., weight 48.2 kg., aged 35, tall and thin. Typhoid of mild type. Admitted to alcoholic service, September 24, 1911, the sixth day of disease. Transferred to medical service, September 26. Discharged, November 8. Fever lasted thirty-one days. No relapse. Patient irrational at times, at first probably from alcoholism. Suffered from severe nausea and vomiting early in disease, probably from alcoholic gastritis. Slight diarrhea to nineteenth day.

Seventeenth day, T. 101-103; 87 food calories per kilogram. Eighteenth to twenty-fourth days, T. 99.8-103.5; from 55 to 83 calories per kilogram. Twenty-fifth day, T. 99.4-102.2; 102 calories per kilogram.

Hugh K., weight 66.8 kg., aged 32, large and muscular. Typhoid fever of very severe type. Admitted Sept. 29, 1911, probably fifth day. Discharged, November 9. Original fever lasted thirty days. No relapse. Took food extremely well. No gastric or intestinal disturbance. Was given whisky till October 10. Not restless.

Twenty-first day of disease, T. 101.2-103; 81 food calories per kilogram. Twenty-second and twenty-third days, T. 99.8-102; from 73 to 92 calories per kilogram. Twenty-fourth day, T. 99-101; 92 calories per kilogram.

Salvatore L., weight 43.9 kg., aged 17, a thin, growing boy. Typhoid fever of mild type. Admitted, Sept. 5, 1911, seventh day of disease. Discharged, October 11. Original fever lasted eleven days. Patient was afebrile for five days and then had a relapse of seven days' duration. Patient suffered from slight nausea at times but for the most part took food well. No restlessness.

Convalescence first to sixth days, from 37 to 76 calories per kilogram in food. Relapse, first and second days, T. 97.6-102; 76 calories per kilogram. Third day, T. 97.8-102; 141 calories per kilogram. Fourth day, T. 98.3-102; *170 calories per kilogram.* Fifth day, T. 98.2-101.6; 125 calories per kilogram. Second convalescence, first to fourth days, from 86 to 94 calories. Fifth to ninth days, from 140 to 166 calories per kilogram.

Alexander C., weight 58.9 kg., aged 36, of medium frame. Typhoid fever, severe type. Admitted July 14, 1911, eighth day of disease. Discharged, October 3. Original fever lasted ten days followed by relapse of thirty days' duration. Complications on admission, severe bronchitis with pleurisy. Took food poorly. Patient suffered from tympanites until well along in disease. Was incontinent of urine and feces much of the time. Irrational at times.

Sixth to tenth days of convalescence, from 86 to 89 food calories per kilogram. Eleventh day, 104 calories per kilogram. Twelfth day, 89 calories per kilogram. Thirteenth to seventeenth days, from 83 to 113 calories per kilogram. Eighteenth day, 78 calories per kilogram. Nineteenth day, 112 calories per kilogram. Twentieth day, 113 calories per kilogram.

Louis M., weight 48.6 kg., aged 22, short and slight. Typhoid fever. Admitted Sept. 7, 1912, the ninth day of the disease. Discharged convalescent, October 30. Type of disease severe, fever lasting twenty-eight days. No relapse. Took food well. No diarrhea or gastric disturbance. Returned in March, 1913, to serve as a normal control.

Seventh to eleventh days of convalescence, from 64 to 69 food calories per kilogram.

Hugo T., weight 63.2 kg., aged 17, tall and muscular. Typhoid fever of severe type. Admitted Aug. 28, 1912, recorded as sixth day of disease but uncertain. Discharged Oct. 25, 1912. Fever lasted to thirty-second day. Delirious early. Did not take food well until steep-curve period. In convalescence took up to 7,000 calories a day. No diarrhea or tympanites. Not restless.

Twenty-first day of disease, T. 101.2-103; 81 food calories per kilogram. Twenty-second and twenty-third days, T. 99.8-102; from 73 to 92 calories per kilogram. Twenty-fourth day, T. 99-101; 92 calories per kilogram.

TABLE 7.—Clinical Data, John H.

Day of Convalescence	Total Calories	Cal., Per kg.	Food			Urine, N.	Feces, N.†	N, Balance	Body Wght., kg.
			Fat, gm.	Carb. gm.	Nitrogen, gm.				
2	3,144	58	265	107	9.7	8.9	1.0	—0.2	
3	2,500	46	179	150	7.9	8.5	0.8	—1.4	54.3
4	4,220	78	216	430	17.2	9.2	1.7	+6.3	54.4
5	4,100	74	219	413	14.6	9.2	1.5	+3.9	55.1
6	3,550	64	181	353	16.2	9.2	1.6	+5.4	55.6
7	2,950	53	26	587	11.9	8.7	1.2	+2.0	55.8
8	3,010	53	26	600	11.1	7.0	1.1	+3.0	56.4
9	3,020	53	26	612	10.3	5.9	1.0	+3.4	56.4
10	2,950	52	26	594	10.3	6.9	1.0	+2.4	56.6
11	3,202	56	129	425	10.2	6.1	1.0	+3.1	56.8
12	2,980	52	26	604	10.3	7.7	1.0	+1.6	57.6
13	3,140	55	119	434	9.8	7.0	1.0	+1.8	57.5

† Assumed 10 per cent. of food N.

The tables of clinical data, Tables 6 to 8, need little explanation. In three cases, John H., Frank W. and Joseph T., the clinical and experimental data have been given in full. In the rest of the cases the lengthy tables have been abbreviated as much as possible. The days of the disease are numbered from the time when the patient first began to feel definitely sick and not from the time he first took to bed, since many typhoid patients take to bed only when the disease is well advanced. The febrile period has been divided, somewhat arbitrarily perhaps, in order to make it possible to compare the different cases. No observations were made in the period of ascending temperature ("First Week"), and only two in the continued temperature period ("Second Week"). During the so-called early steep-

TABLE 8.—CLINICAL DATA, JOSEPH T.

Day of Disease	Min. Temp.	Max. Temp.	Food, Total Cal.	Food, Cal. per Kg.	Food, Fat, Gm.	Food, Carb., Gm.	Food, N., Gm.	Urine, N.	Feces, N.*	N, Balance	Body Wght., Kg.
(Early Stee	p-Curve Per	iod)									
30	101.2	103.0	1,940	33	115	165	7.7				
31	101.0	102.4	2,200	38	141	156	9.7				
32	101.0	103.0	3,820	65	209	355	16.6	12.3	1.7	+ 2.6	58.4
33	100.4	102.2	3,530	60	219	256	17.5	16.3	1.8	— 0.6	
34	99.8	103.0	4,080	70	135	614	12.2				
35	100.0	102.2	4,350	74	188	525	17.2	....	....	....	58.9
(Late Steep	-Curve Peri	od)									
36	99.4	102.0	3,940	67	172	474	15.5				
37	99.2	101.8	4,450	75	193	534	18.2	....	....	....	58.9
38	99.4	101.8	4,120	70	182	491	16.2				
39	99.0	100.2	5,330	90	192	741	19.8	....	....	....	59.2
40	98.4	100.0	3,690	62	158	447	15.1				
(Convale	scence)										
1	98.0	99.2	6,240	05	319	655	23.0				
2	98.2	99.0	4,970	82	252	523	18.8	....	....	....	60.7
3	98.2	99.0	5,700	94	272	615	25.2	11.4	2.5	+11.3	
4	98.0	99.0	5,380	87	265	598	18.0	9.4	1.8	+ 6.8	61.8
5	97.8	99.1	5,360	87	267	593	17.3				
6	98.0	99.2	5,500	89	292	518	25.7	....	....	....	61.8

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* 10 per cent. of food nitrogen.

curve period ("Third Week"), when the temperature made sharp remissions but never touched normal, twenty-one experiments were recorded. In the late steep-curve period ("Fourth Week"), with a temperature dropping to normal almost every morning, there were thirty-three observations; five observations were made during a relapse. During convalescence, the days are numbered from the first day of normal temperature, and the results grouped in actual weeks of seven days. In 1911 the twenty-four-hour specimens of urine were collected by the head nurse personally, preserved with thymol and chloroform and analyzed the day the specimen ended. In 1912 each voiding was kept separate and preserved in an ice-box with the addition of toluene. The voidings for the whole day were either united or analyzed separately if it were necessary to fractionate the urine. Nitrogen determinations were made by the Kjeldahl method, glucose

TABLE 9.—Respiration—

Day of Disease	Start of Experiment	Recent Food				During Experiment		
		Time	Fat, gm.	Carb., gm.	Prot., gm.	Temp.	Average Pulse	Average Resp.
(Contin.	Temp.)							
11	p. m.	9:00 a.m.	8	12	15			
	2:38†	1:30 p.m.	7	11	14	103.6	111	27
14	....	9:00 a.m.	12	33	2			
	2:27	1:00 p.m.	..	70	..	103.2	112	26
(Early Steep-	Curve Period)							
16	....	9:00 a.m.	25	42	3			
	2:57	11:00 a.m.	..	70	..	103.0	100	22
18	....	12:00 m.	37	42	25			
	3:17	2:30 p.m.	4	12	2	102.8	104	24
21	....	12:00 m.	33	29	24			
	3:41	2:30 p.m.	17	10	7	103.0	104	18
22	3:06	9:00 a.m.	17	10	7	102.8	115	17
(Late Steep-	Curve Period)							
24	....	12:00 m.	37	28	25			
	3:06	2:30 p.m.	17	10	7	100.2	105	16
28	....	12:00 m.	43	30	26			
	3:25	* 2:30 p.m.	23	12	7	99.8	118	19
30	....	12:00 m.	43	30	26			
	2:33	2:20 p.m.	17	10	7	99.0	107	18
§	3:30	....	..	...	..	99.0	107	16

* Unusually low total nitrogen in urine on this day.

† First experiment with this patient.

by the methods of Stanley R. Benedict[28] and creatinin by Folin's[29] method. In 1912 these analyses were made by Mr. Gephart. The cases in which the stools were analyzed, Charles S. and Frank R., have been described in a previous publication.[2] In all other cases the fecal nitrogen was estimated at 10 per cent. of the food nitrogen. The patients were weighed as frequently as possible by means of the scales previously described.[30]

EXPERIMENTS, FRANK W.

Per Kg. and Min.		R. Q.	Calculated Heat Production					Food for 24 Hours, Cal. Per Kg.
O_2, c.c.	O_2, c.c.		Per Cent. Calories from			Cal. Per Sq. M., Per Hr.	Cal. Per Kg. Per 24 Hours	
			Prot.	Fat	Carb.			
4.35	5.48	0.79	13.7?*	....	...	48.3	37.6	39
4.48	5.22	.86	25	29	46	46.0	35.8	35
4.10	5.33	.77	19	65	16	46.1	35.9	46
4.93	5.71	.86	25	28	47	50.0	39.2	71
4.25	4.89	.87	29	23	48	42.5	33.5	50
3.86	5.01	.77	19	64	17	42.8	33.7	35
3.94	5.10	.77	24	61	15	43.3	34.2	76
4.67	5.71	.82	22	46	32	49.5	38.8	76
4.63	4.87	.95	15‡	..	..	44.2	34.7	68
3.92	4.58	.86	15‡	..	..	40.7	3.19	

‡ Estimated. § *Auspumpung* in all subsequent experiments.

The calculation of the urinary nitrogen for the fifteen-minute period is necessarily somewhat inaccurate; no method is entirely satis-

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[28] Benedict. Stanley R.: The Detection and Estimation of Glucose in Urine, Jour. Am. Med. Assn., 1911, lvii, 1193.

[29] Folin. O.: Beiträge zur Chemie des Kreatinins und Kreatins im Harne, Ztschr. f. Physiol. Chem., 1904. xli. 223.

[30] Coleman: Diet in Typhoid Fever, Jour. Am. Med. Assn., 1909, liii, 1145.

TABLE 10.—Respiration Experiments,—

Day of Disease	Start of Experiment	Recent Food				During Experiment		
		Time	Fat, gm.	Carb., gm.	Prot., gm.	Temp.	Average Pulse	Average Resp.
Joseph T.:								
(Early Steep-	Curve Period)							
31	....	12 m.	17	24	11			
	3:02 p. m.	2:45 p.m.	18	16	8	102.2	83	15
(Late Steep-	Curve Period)							
36	2:51 p. m.	12:00 m.	42	59	29	101.4	83	16
	4:09 p. m.	......	..	..	..	102.0	78	14
38	2:17 p. m.	12:00 m.	48	66	35	101.0	83	18
	3:41 p. m.	2:36 p.m.	..	125	..	101.8	85	21
39	....	12:00 m.	38	112	21			
	3:46 p. m.	2:16 p.m.	..	125	8	100.2	80	17
	4:22 p. m.	......	..	..	..	100.2	78	19
(Convales	cence)							
2	....	9:00 a.m.	38	47	17			
	3:21 p. m.	11:00 a.m.	..	125	..	99.0	73	16
	4:17 p. m.					99.0	74	15
4	....	9:00 a.m.	46	70	21			
	3:19 p. m.	11:00 a.m.	..	125	..	98.3	70	17
	4:17 p. m.‡	......	..	..	..	98.3	70	17
5	....	11:00 a.m.	..	125	..			
	4:04 p. m.	1:00 p.m.	46	115	15	99.2	93	16
6	....	12:00 m.	43	91	39	..	..	..
	3.47 p. m.	2:00 p.m.	18	69	8	98.2	81	15
		......	..	..	..	98.2	82	16
John H.:								
(Convales	cence)							
3	10:41 a. m.*	4:30 p.m*	62	28	16	98.5	66	15
11	....	5, 6, 7, 8, 9,						
	10:13 a. m.	10:00 a.m.	0	28	3	98.4	71	15
	10:49 a. m.	......	..	..	..	98.4	76	15
11	....	11, 12, 1, 2,						
	4:00 p. m.	3:00 p.m.	11	28	3	98.4	82	10
13	10:10 a. m.	10:00 a.m.	0	28	3	98.6	77	12
	10:54 a. m.†	......	..	..	..	98.6	74	12
	3:12 p. m.	3:00 p.m.	11	28	3	98.6	82	12
	4:03 p. m.	......	..	..	..	98.6	78	12

* Eighteen hours after last food.

† Seventeen-minute period.

—Joseph T. and John H.

Per Kg. and Min.		R. Q.	Calculated Heat Production					Food for 24 Hours, Cal. Per Kg.
			Per Cent. Calories from			Per Sq. M. Per Hour	Cal. Per Kg. Per 24 Hours	
CO$_2$, c.c.	O$_2$, c.c.		Prot.	Fat	Carb.			
4.07	5.42	0.75	..	..	..	48.3	36.7	38
4.07	5.32	.77	..	..	..	47.8	36.2	67
4.02	5.13	.78	..	..	..	46.3	35.1	
4.53	5.36	.85	..	..	..	49.2	37.2	70
4.56	5.08	.90	..	..	..	47.1	35.8	
4.03	4.57	.88	..	..	..	42.1	32.0	90
3.86	4.04	.96	..	..	..	38.1	28.9	
3.74	4.25	.88	..	..	..	39.6	29.8	82
3.77	4.03	.94	..	..	..	38.1	28.6	
3.95	3.97	1.00	14	0	86	38.9	29.1	87
3.55+	3.55—	1.02	15	0	85	35.0	26.1	
4.74	5.59	.85	..	..	..	52.0	38.9	87
4.98	6.16	.81	..	..	..	56.7	42.4	89
4.95	5.30	.93	..	..	..	49.0	37.6	
2.83	3.92	.72	16	83	1	33.7	26.3	46
4.11	4.05	1.02	10	0	90	38.1	29.3	52
4.24	3.82	1.11	10	0	90	36.4	28.0	
4.52	5.06	.89	8	31	61	46.3	35.6	
4.51	4.09	1.10	11	0	89	39.2	29.9	55
4.33	4.23	1.03	11	0	89	40.1	30.6	
4.78	4.56	1.05	10	0	90	43.4	33.1	
4.95	4.90	1.01	9	0	91	46.3	35.4	

‡ Twenty-minute period.

TABLE 11.—RESPIRATION EXPERIMENTS, CHARLES—

Day of Disease	Start of Experiment	Recent Food				During Experiment		
		Time	Fat, gm.	Carb., gm.	Prot., gm.	Temp.	Average Pulse	Average Resp.
Charles S.:								
(Early Steep-	Curve Period)							
10	....	12:30 p.m.	33	75	25			
	3:16 p. m.	2:30 p.m.	..	125	..	99.4	66	30
	3:57 p. m.	....	..	...	..	99.4	66	19
12	....	7:00 a.m.	18	70	10			
	11:41 a. m.	9:00 a.m.	13	52	8	100.2	58	18
	1:01 p. m.	1:35 p.m.	12	53	2	100.2	61	..
	2:37 p. m.	....	..	...	..	100.2	61	18
(Late Steep-	Curve Period)							
15	....	12:00 m.	58	132	47			
	3:25 p. m.	2:30 p.m.	13	52	8	101.2	64	16
	4:06 p. m.	....	..	...	..	101.2	65	17
(Relapse)								
20	....	6:45 p.m.	13	32	8			
(Second Day	of Relapse)							
	9:30 a. m.	7:00 a.m.	2	43	2	100.0	74	21
	10:35 a. m.	9:55 a.m.	12	53	2	100.0	72	19
	12:10 p. m.	....	..	...	..	100.2	78	17
William M.:								
(Early Steep-	Curve Period)							
23	....	12:00 m.*	58	119	42			
	4:11 p. m.	2:30 p.m.	18	30	8	102.2	89	17
24	....	12:00 m.*	74	99	45			
	3:42 p. m.	2:30 p.m.	35	78	14	102.8	97	17
25		12:00 m.*	72	130	45			
	2:36 p. m.	2:29 p.m.	25	95	12	101.5	92	17
	3:27 p. m.	....	..	...	..	102.0	90	14
(Late Steep-	Curve Period)							
31	....	9:00 a.m.	67	76	31			
	2:41 p. m.	12:00 m.*	64	93	49	100.0	97	14
	3:31 p. m.	....	..	...	..	100.0	95	16
	4:31 p. m.	....	..	...	..	100.2	90	15
32	9:00 a. m.	9:00 a.m.	35	68	14			
	2:52 p. m.	12:00 m.	73	121	52	100.3	91	16
Archie C.:								
(Early Steep-	Curve Period)							
17	....	11:45 a.m.	28	24	23			
	3:47 p. m.	3:07 p.m.	..	125	..	102.7	100	22
25	....	12:00 m.	44	82	27			
	4:22 p. m.†	2:30 p.m.	6	50	3	100.6	94	15
Hugh K.:								
(Early Steep-	Curve Period)							
21	....	12:00 m.	43	71	19	102.8	106	14
24		12:00 m.	65	96	44			
	3:23 p. m.	2:30 p.m.	..	75	..	100.4	109	18

* Whisky, 15 c.c., with noon meal.

—S., WILLIAM M., ARCHIE C. AND HUGH K.

Per Kg. and Min.		R. Q.	Calculated Heat Production		Food for 24 Hours, Cal. Per Kg.	Body-Weight, kg.
CO_2, c.c.	O_2, c.c.		Cal. per Sq. M. per Hour	Cal. Per Kg. Per 24 Hours		
4.41	4.82	0.92	44.8	34.1	75	
4.44	4.83	0.92	44.8	34.1		
3.94	4.47	0.88	41.2	31.4	49	
3.87	4.23	0.91	39.1	29.9		
4.02	4.46	0.90	41.2	31.4		
4.41	4.78	0.93	44.4	33.8	77	57.7
4.49	5.04	0.89	46.5	35.4		
3.89	4.70	0.83	42.5	32.4	67	58.6
3.86	4.76	0.81	43.2	32.8		
4.11	4.67	0.88	42.8	32.6		
4.56	5.09	0.90	49.2	35.8	89	
5.15	5.55	0.93	54.2	39.4	92	67.3
4.66	4.91	0.95	48.3	35.0	83	68.2
4.85	5.09	0.95	50.0	36.3		
4.93	5.16	0.96	51.0	36.8	76	68.6
4.86	5.04	0.96	50.2	36.2		
4.60	4.71	0.98	46.9	33.8		
4.49	4.77	0.94	47.1	34.0	66	69.3
4.38	5.33	0.82	44.8	36.4	87	
4.56	4.96	0.92	43.6	35.1	102	49.1
5.18	5.73	0.90	55.4	40.1	81	66.9
4.99	5.36	0.93	52.1	38.0	92	67.0

† Nine-minute period.

factory. A few well-trained patients were able to void every two hours, and the calculations are based on such fractional specimens. In most cases the twenty-four-hour specimen was used because it

TABLE 12.—

Day of Disease	Time After Last Meal, Hrs., Min.	Temperature During Experiment	Per Kg. and Min.	
			CO_2, c.c.	O_2, c.c.
Salvatore L.:				
(Last Day of	Fever)			
10	2-57	99.6	4.57	6.10
	3-46	99.6	4.48	6.16
(Relapse)				
4	1-23	102.2	6.62	6.32
	1-57	102.2	6.37	6.53
5	3- 7	100.2	5.47	6.34
	0-31	100.2	5.37	5.83
	1-17	100.2	5.85	5.81
(Second Con	valescence)			
6	1-58	98.2	6.43	6.12
7	2-39	98.4	5.70	5.98
	0.43	98.4	6.17	6.06
	1-37	98.0	6.01	6.27
9	0-41	98.0	6.12	5.87
	1-20	98.0	6.05	5.65
John C.:				
(Early Steep-	Curve Period)			
35	0-15	101.0	3.38	4.30
39	7-35	103.2	3.36	4.71
Abe B.:				
(Late Steep-	Curve Period)			
37	3- 0	100.2	3.75	3.95
	3-49	100.5	3.88	4.14
39	1-05	99.2	5.77	6.56
	1-54	99.4	5.70	6.22
43	1-26	99.6	3.98	4.09
	2- 0	99.8	3.82	3.82
Anton V.:				
(Late Steep-	Curve Period)			
33	2-51	100.0	3.69	4.55
(Convales	cence)			
3	2-33	98.4	3.35	3.89
	1-30	98.4	3.37	3.70
	3-28	98.4	3.28	3.82
(Relapse)				
7	7-56	101.6	3.80	4.65
	0-49	101.8	4.32	5.41
(Second Con	valescence)			
10	2-28	98.0	4.62	4.69
	3-43	98.0	4.71	4.55
	4-56	98.0	3.95	4.06
21	3-42	98.0	4.64	4.81

seemed fair to assume that with patients on a liberal diet of moderate nitrogen content, fed at frequent intervals, the protein metabolism would not vary greatly from hour to hour.

In most of the tables of the respiration experiments (Tables 9 to 11), the composition of all food administered within the five or six hours preceding the observations with the Benedict machine has been given, since different meals affect metabolism in different ways. In all cases in which the meal consisted of carbohydrate alone, nothing but lactose in lemonade was given. In the abbreviated tables (Tables

-RESPIRATION EXPERIMENTS

R. Q.	Heat Production		Food for 24 Hours, Cal. Per Kg.	Weight
	Cal. Per Kg. Per 24 Hrs.	Cal. Per Sq. M. Per Hour		
.75	41.4	49.5	73	43.9
.73	41.5	49.6		
1.04	48.7	58.3	170	44.3
.97	46.7	55.8		
.86	43.9	52.5	125	
.92	40.9	49.2		
1.01	43.1	51.7		
1.05	44.0	53.5	149	
.95	42.7	52.1	166	46.1
1.02	43.7	53.2		
.96	44.8	54.6		
1.04	45.0	55.0	142	47.3
1.07	44.5	54.4		
.79	29.4	39.6	54	62.3
.70	31.7	42.5	53	62.7
.94	28.0	39.9	55	74.8
.95	29.2	41.7		
.88	45.9	65.8	64	
.92	43.9	62.9		
.97	29.2	41.7	48	74.5
1.00	28.1	40.1		
.81	29.8	39.9	40	62.3
.86	27.1	36.2	51	61.1
.91	26.1	34.7		
.86	26.6	35.4		
.82	32.1	46.7	47	59.8
.80	37.1	49.2		
.99	33.6	45.2	108	61.8
1.04	32.6	43.8		
.98	29.8	39.9		
.97	34.5	47.1	....	65.5

12 to 14), these data have been omitted for the sake of brevity. When the non-protein respiratory quotient was over 1, it has been assumed that carbohydrate was being converted into fat and the extra heat due to this has been calculated by the method described by Lusk,[31] in which 1 liter of excess of carbon dioxid over and above the volume eliminated, when the respiratory quotient is unit, is given the value of 0.803 calories.

TABLE 13.—

Day of Convalescence	Time After Last Meal, Hrs., Min.	Temperature During Experiment	Per Kg. and Min.	
			CO_2, c.c.	O_2, c.c.
Louis M.:				
7	0-46	99.2	5.75	5.58
	1-38	99.2	5.60	5.47
10	2-30	99.8	4.84	5.94
11	0-58	99.2	5.00	5.97
Hugo T.:				
24	1-18	98.2	5.11	5.54
26	14-26	97.8	3.29	4.16
	0-30	98.4	5.37	5.47
	1-16	98.4	5.03	5.06
	2- 7	98.4	5.13	5.18
32	17-30	98.0	3.42	4.11
	1- 9	98.4	3.53	4.22
Frank R.:				
3	0-43	98.0	4.96	5.10
5	3-34	98.2	4.92	4.81
Alex C.:				
6	0-42	98.4	4.64	5.03
	1-46	98.0	4.60	5.22
10	0-35	98.8	4.61	5.02
	1-38	99.2	4.30	4.70
12	1-38	99.0	4.47	4.70
18	5-42	99.0	4.48	4.62
20	0-44	99.8	4.78	5.23
	1-26	99.8	4.99	5.26
Davide G.:				
(Early Steep-	Curve Period)			
27	0-41	102.0	5.98	7.17
(Late Steep-	Curve Period)			
30	4-33	101.8	5.08	6.38
	5-23	101.8	5.04	6.53
32	2-17	100.2	4.62	5.51
	3-32	100.2	4.74	5.57
33	1-13	100.2	5.30	6.11
	2- 9	100.2	5.20	5.73
37	0-56	100.0	5.10	5.72
	1-39	100.0	4.94	5.48

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[31] Williams, Riche and Lusk: Animal Calorimetry, Second Paper, Metabolism of the Dog Following the Ingestion of Meat in Large Quantity, Jour. Biol. Chem., 1912, xii, 358.

DISCUSSION OF RESULTS

As has been stated, the present investigation was undertaken primarily to discover the fate and general influence on metabolism of the large amount of food which is given to typhoid-fever patients in this clinic. So many factors enter into the figures appearing in the tables that it is difficult to classify the results. Moreover, most of the statements in the following discussion have been based on the averages obtained in the different periods of the disease, and it must be remembered that the limits of variation may be considerable as is shown in Figures 1 and 2.

—Respiration Experiments

R. Q.	Heat Production		Food for 24 Hours, Cal. Per Kg.	Weight, Kg.
	Cal. Per Kg. Per 24 Hrs.	Cal. Per Sq. M. Per Hour		
1.03	40.5	50.0	64	48.6
1.02	39.7	49.0		
.81	40.8	50.2	68	
.84	41.3	51.0	68	48.4
.92	39.2	52.8	87	63.2
.79	28.5	38.4	54	63.6
.98	39.4	53.1		
.99	36.5	49.2		
.99	37.3	50.2		
.83	28.2	37.9	29	62.5
.84	29.2	39.2		
.97	36.6	48.8	99	61.6
1.02	34.6	46.3	66	
.92	35.6	47.1	88	58.9
.88	36.6	48.3		
.92	35.4	47.1	86	59.5
.91	33.2	44.2		
.95	33.5	44.7	89	59.9
.97	33.1	44.7	78	
.91	36.9	50.0	113	63.6
.95	37.5	50.6		
.84	49.3	64.2	63	56.1
.80	43.3	56.3	71	55.7
.77	44.1	57.1		
.84	37.9	49.2	76	56.0
.85	38.4	49.8		
.87	42.4	55.0	88	55.2
.91	40.1	51.9		
.89	40.2	51.9		
.90	38.6	50.0		

The question of varying weights is fairly easily disposed of. The average weights of the high-calory cases investigated during the febrile period was 59.4 kg., and during convalescence 56.3 kg. The average weight of the cases of Kraus, Svenson, Grafe and Rolly which have been used for comparison was 53.7 kg. in the febrile period and 50.4 in convalescence. According to the table of normal controls this difference of about 6 kg. would make the lighter patients show an increase of only 2 or 3 per cent. in metabolism when the calories were calculated according to body-weight. The metabolism of both the high-calory and fasting groups of typhoid cases can therefore be compared with the normals whose weights were between 50 and 60 kg. and whose heat production averaged 26.5 calories per kilogram.

TABLE 14.—Respiration Experiments with "Auspumpung" (Carbon Dioxid and Respiratory Quotient Probably Too High on Account of Increasing Ventilation)

Day of Convalescence	Start of Exper.	Per Kg. and Min.		R. Q.	Cal. per Kg. for 24 Hrs.	
		CO_2, c.c.	O_2, c.c.		Produced Calculated from O_2	Food
Frank W.:						
1	2:46 p. m.	4.85	5.23	.93	36.7	73
	3:21 p. m.	5.17	5.21	.99	38.2	
4	2:38 p. m.	5.84	5.22	1.12	42.9	83
	4:22 p. m.	4.90	4.36	1.13	36.1	
6	9:49 a. m.*	4.02	4.15	.97	29.6	68
	10:22 a. m.	4.77	4.36	1.09	35.1	
	2:47 p. m.	5.67	5.11	1.10	41.7	
	3:29 p. m.	5.58	5.21	1.07	41.0	
8	9:47 a. m.*	5.44	4.69	1.16	39.9	71
Louis M.:	2:47 p. m.	6.61	5.82	1.14	48.5	
8	2:35 p. m.	6.50	6.22	1.05	45.3	65
	3:34 p. m.	6.47	5.48	1.18	40.7	
19	9:34 a. m.*	5.23	5.18	1.01	37.2	37
	11:16 a. m.	5.78	5.06	1.14	37.1	
	1:28 p. m.	5.52	4.77	1.16	35.3	
	3:15 p. m.†	5.40	4.98	1.08	36.4	
23	10:02 a. m.	5.16	4.77	1.08	34.8	40
	2:35 p. m.	6.00	4.94	1.22	36.8	
	3:41 p. m.	5.37	4.68	1.15	34.4	

* Seventeen hours after food. † Twenty minutes after period.

Table 15 gives a direct comparison of the averages of the fasting and the high-calory cases in the different stages of the disease and convalescence. As with the normal controls, the metabolism of the fasting patients was calculated from the average oxygen consumption and the average quotient. The tables of Magnus-Levy were used, and although the protein furnished more than 15 per cent. of the total calories, it might have furnished 20 per cent. without making a difference of 1 per cent. in the caloric value of the oxygen. The calories per kilogram of the groups of high-calory cases are calculated in the same manner in one column, and next to it are given the averages of calculations made according to the more accurate method of Loewy, using the urinary nitrogen. The results of Kraus, Svenson, Grafe and Rolly have been recalculated according to calories per square meter of body-surface per hour for comparison with the high-calory cases. In the last two columns of the table a percentage comparison has been made showing that in the steep-curve period of typhoid fever the liberally fed patients have a metabolism from 36 to 40 per cent. higher than normal, while the fasting patients show an increase of 23 or 24 per cent. During convalescence the relation of the high-calory cases to normal is about the same, but the increase over the fasting patients is much greater. It is unfortunate that we have been unable to study a series of typhoid patients on low diets, but we have not felt it justifiable to withhold food. For this reason it has been necessary to use the work of others as a basis of comparison. The somewhat lower metabolism of the fasting cases of other observers may have been due to the low state of nutrition.

HEAT PRODUCTION

Febrile Period.—Since only two observations were made on the high-calory cases in the period of continued temperature, a percentage comparison of their heat production with that of the fasting cases would not be justified.

In the early steep-curve period the high-calory cases show an increase of 5 per cent. in metabolism over fasting patients, calculated according to body weight; in the late steep-curve period the increase amounts to only 2 per cent. Calculated according to surface area, the percentage increases are 11 and 13 per cent., respectively.

The absence of a greater increase in metabolism when patients were taking from 60 to 100 and more food calories per kilogram per day directly raises the question whether or not food exhibits a specific dynamic action in fever. This slight increase in metabolism is all the more noteworthy since the fasting patients were studied in the early morning when, it may be assumed, the temperature and metabolism were lowest for the day, while the high-calory patients were studied in the afternoon, usually shortly after food.

That the total metabolism of normal subjects is raised after the taking of food, and particularly after protein food, has been proved repeatedly by the work of Voit,[32] Rubner,[33] Magnus-Levy,[34] Lusk,[31, 35] Howland[36] and many others. Recent studies by Lusk[37] have shown that, in the case of protein, this is due to the stimulus of the amino-acids, and in the case of fat and carbohydrates to a plethora of fat and carbohydrate metabolites in the circulation.

These results of Lusk's confirm Rubner's observation that the specific dynamic action of a mixed diet does not represent the sum of the specific dynamic actions of the individual foodstuffs in the diet, but is considerably less than this.

TABLE 15.—Table of Averages: High-Calory Cases Compared with Normal—Grafe and—

	Number of Observations		O_2 c.c. Per Kg. and Min.		R. Q.	
	Fasting	High Calory	Fasting	High Calory	Fasting	High Calory
Normal controls, weight 50-60 kg.	17	..	3.86	....	...	....
Typhoid patients, continued temperature period	11	2	5.33	5.35	.78	0.83
Early steep-curve period...	19	21	4.98	5.11	.76	0.87
Late steep-curve period....	21	32	5.23	5.20	.76	0.88
Convalescence, first week..	25	21	3.90	4.98	.82	0.93
Convalescence, second week	16	17	4.08	4.85	.84	1.01
Convalescence, third week	15	4	4.19	5.00	.92	0.95
Convalescence, fourth and fifth weeks	24	7	4.30	4.85	.96	0.91
Several months after recovery	10	..	3.85	....	.81	....

* Reckoned from calories per square meters body-surface.

† 12 Normal controls, weights from 50 to 83 kg. in bed calorimeters.

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[32] Voit, Carl: Physiologie des Stoffwechsels und der Ernährung, 1881, pp. 308, 311.

[33] Rubner: Die Gesetze des Energieverbrauchs, 1902.

[34] Magnus-Levy: Ueber die Grosse des respiratorischen Gasewechsels unter dem Einfluss der Nahrungsaufnahme, Arch. f. d. ges. Physiol. (Pflüger's), 1894, lv, 1.

[35] Lusk: Third Paper, Metabolism After the Ingestion of Dextrose and Fat, Including the Behavior of Water, Urea and Sodium Chlorid Solutions, Jour. Biol. Chem., 1912, xiii, 27; Sixth Paper, The Influence of Mixtures of Foodstuffs on Metabolism, ibid., 1912, xiii, 185.

[36] Howland: Der Chemismus und Energieumsatz bei schlafenden Kindern, Ztschr. f. Physiol. Chem., 1911, lxxiv, 1.

[37] Lusk: Fifth Paper, The Influence of the Ingestion of Amino-Acids on Metabolism, Jour. Biol. Chem., 1912, xiii, 155; The Cause of the Specific Dynamic Action of Protein, The Archives Int. Med., 1913, xii, 485, and the references given in Footnote 35.

While our study of the specific dynamic action of food in typhoid fever is not yet completed, it is clear, from the figures in the tables, that, compared with fasting cases, the high-calory patients, even when taking enormous amounts of food, exhibit only a slight, if not negligible, increase in heat production, and we would suggest, tentatively, as the explanation that the stimulation of metabolism by the toxic agent of the disease is so intense that further stimulation by food is insignificant. This would be analogous to the absence of summation of the specific dynamic actions of foodstuffs in a mixed diet or to the absence of specific dynamic action when the heat production is increased through subjection of a normal individual to the influence of cold.

—Controls and with Fasting (Nüchtern) Low-Calory Cases of Kraus, Svenson,
—Rolly

Cal. per Kg. per 24 Hours	Cal. per Kg. per 24 Hours High Calory		Cal. per Square Meters Body Surface		Percentage Increase Above* Normal Controls	
Fasting	Calculated from N. of Urine	Calculated from Av. O_2 and Av. R. Q.	Fasting	High Calory	Fasting Patients	High Calory Patients
26.5	....	....	34.2 †			
36.4	35.9	37.0	47.3	47.2	38	37
33.9	35.6	35.7	42.4	46.9	23	36
35.6	36.3	36.0	42.6	48.0	24	40
26.9	35.8	34.9	34.0	45.3	— 1	32
28.3	37.0	36.0	35.9	45.0	4	31
29.7	36.2	35.7	38.4	48.1	12	40
30.6	35.9	35.4	38.0	45.8	11	33
26.5	....	....	39.9			

Clinically the observation is important in that it proves unfounded the fear that large amounts of food in the febrile period of typhoid may cause too great an increase in heat production and, in turn, a rise of the fever temperature, thus confirming by scientific experiment the early clinical belief of von Hoesslin,[38] that large quantities of ingested food were without influence on the temperature or heat production in fever.

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[38] Von Hoesslin: Experimentelle Beiträge zur Frage der Ernähung fiebernder Kranker, Virchow's Arch. f. path. Anat., 1882, lxxxix, 95, 303; Ueber den Einfluss der Nahrungszufuhr auf Stoff und Kraftwechsel, ibid., p. 333.

Convalescence.—Throughout convalescence — at least into the fourth week—the metabolism of the liberally fed patients is just as high as during the fever. Yet the returning control of the heat-regulating mechanism permits rapid dissipation of the excess of heat, and the temperature remains normal. The reason for the continuance of the high metabolism into convalescence is not clear. It may be that, as the stimulation of metabolism, which the disease causes, disappears, the specific dynamic action of the food again becomes manifest; the convalescing organism may be doing its work uneconomically, as Svenson believes, or the basal metabolism of the typhoid convalescent may be high, as Howland and others have shown to be the case with normal growing infants. The solution of the problem must be left for future investigation.

TABLE 16.—Food Required to Bring Typhoid Patients Into Nitrogen Equilibrium

Name	Day of Disease	Range of Max. Temp.	Average Food Cal. Per Kg. Per 24 Hours	Average Food N. Per 24 Hours	Average N. Balance Per 24 Hrs.
R. N.*	20-24	104.4-102.0	71	10.9	+1.5
U. H.*	27-30	100.4-101.0	68	8.9	+0.4
Z. O.*	9-12	104.0-103.2	72	13.9	—0.2
Z. O.*	13-18	103.6-102.8	85	15.0	+0.6
Charles N.†..	28-31	102.0-101.8	69	18.7	+6.0
Michael K.†..	15-17	102.0-100.5	79	17.9	+1.5
Philip R.† ...	14-15	102.0-102.0	67	17.6	+2.7
John N.† ...	26-30	102.0-104.0	58	17.8	+1.9
Frank W.‡...	24-28	101.0- 99.6	74	20.3	+4.9
F. Ma.§	6- 8	103.1-103.6	45.5	10.5	—0.5
J. Gerl ¶	9-10	103.6-103.8	59.5	7.5	+0.4
J. Gerl ¶	16-21	100.9-103.4	76.6	8.1	+0.03

* Shaffer and Coleman (Footnote 1.) † DuBois (Footnote 2). ‡ This article (Table 6). § Rolland (Footnote 41). ¶ Rolland (Footnote 41) Boy aged 10, weight 26 kg.

The fasting convalescents of the earlier investigations[39] have a total heat production which is only slightly greater than normal until the third week, when there occurs an increase of from 12 to 18 per cent.

Relapse.—Three patients were studied during relapses. They showed an average heat production of 39 calories per kilogram. The average is high on account of the high metabolism of the boy Salvatore. The average for the other two cases is 36.6 calories per kilogram, or essentially the same as the general average in the original fever.

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[39] Rolly, F.: Experimentelle Untersuchungen über den Stoffwechsel im Fieber und in der Reconvalescenz, Deutsch. Arch. f. klin. Med., 1911, ciii, 93; Grafe, E.: Untersuchungen über den Stoff- und Kraftwechsel im Fieber, ibid., 1911, ci, 209.

In this connection it should be pointed out that a large excess of energy over the actual heat production must be supplied to the typhoid patient in order to maintain weight and nitrogen equilibrium. The influence of the high-calory diet on the weight of the patient has been considered in previous publications.[40] It is sufficient to state here that loss of weight may be prevented by giving the patient sufficient food.

The few reported cases in which nitrogen equilibrium was obtained have been grouped in Table 16. With the exception of one patient of Rolland's[41] (who almost reached balance on 45.5 calories), these cases were not brought into equilibrium until the patients were given from 60 to 85 calories per kilogram, and even with the larger amount of food, equilibrium was not established until the temperature had begun to fall. It is evident, therefore, that more than 85 calories per kilogram may be required by some patients to establish nitrogen equilibrium during the height of the fever.

Rolland has estimated 49 calories per kilogram as the mean heat production of the typhoid-fever patient. Basing her calculation on Grafe's[11] average of 35 calories per kilogram for fasting typhoids, she allowed from 10 to 15 per cent. for the specific dynamic action of food, from 10 to 15 per cent. for the excreta, and from 10 to 15 per cent. for muscular work in moving about the bed. Experimental work in this clinic has shown that her allowance for the first two factors is too liberal. It has already been pointed out that the specific dynamic action of the food in the high-calory cases is almost, if not entirely, masked by the increase in metabolism caused by the toxic agent, and it has been shown that the average loss of calories in the feces amounts to 5 per cent. of the intake. Though it is difficult to estimate accurately the increase in metabolism from bodily activity, yet in the high-calory cases it cannot be great—possibly not so great as in patients who are undernourished. The high-calory cases are not "tubbed," and are spared the shivering which ordinarily follows this practice. With rare exceptions they are not toxic and, therefore, not restless, and do not exhibit jactitation and subsultus tendinum. For the most part the patients lie quietly in bed, often reading for several hours a day. Without taking into consideration the reduction of metabolism during sleep, we believe that the increase in metabolism caused by bodily movement is amply covered by an allowance of 10 per cent.

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[40] Coleman: The High-Calory Diet in Typhoid Fever, a Study of 111 Cases, Am. Jour. Med. Sc., 1912, cxliii, 77; Weight Curves in Typhoid Fever, ibid., 1912, cxliv, 659; Diet in Typhoid Fever, Jour. Am. Med. Assn., 1909, liii, 1145.

[41] Rolland, Anne: Zur Frage des toxogenen Eiweisszerfalls im Fieber des Menschen, Deutsch. Arch. f. klin., Med., 1912, cvii, 440.

Making this allowance, the average total heat production of the typhoid patient is 40 calories per kilogram per day.

If the supply of nitrogen be sufficient, a normal man may be maintained in nitrogen and weight equilibrium on a diet which just covers his heat production. It is clear, however, from Table 16 that typhoid patients, though producing an average of 40 calories per kilogram, cannot be brought into equilibrium unless they receive from 57 to 81 calories[42] and in some instances more. In other words, a patient of 65 kg., producing 2,400 calories a day, requires from 3,600 to 5,000 calories to maintain him in equilibrium.

The fate of this excess is a matter of great interest. There can be no doubt that the nitrogen of the urine furnishes an accurate index of protein metabolism in fever. The work of Magnus-Alsleben[43] and Mohr[44] proved that there is no abnormal loss of calories in carbon-containing compounds in the urine except in cases with glycosuria. Glycosuria has been observed but seldom in the Bellevue cases. It is difficult, but not impossible, to conceive that patients are able to store body-fat while there is a negative nitrogen balance and a steady loss of body-weight. A moderate storage of fat could, of course, be masked by a change in the water content of the body, but we are not prepared to offer this theory.

With the onset of convalescence, the amount of food required to maintain nitrogen equilibrium more nearly approaches the estimated heat production of the patient. For example, one of the patients reported by Shaffer and Coleman showed a positive nitrogen balance from the fifth to the ninth days of convalescence while receiving 37 calories per kilogram, yet a few days previously, during a relapse, 77 calories per kilogram were required. F. Müller[45] has reported a case which retained nitrogen from the third to the tenth days of convalescence from typhoid fever on a diet furnishing only 1,165 calories and 9.9 gm. of food nitrogen, which, on account of its low fuel value, would be incapable of maintaining nitrogen equilibrium in health.

These facts demonstrate that the need of the typhoid patient for the excess of energy over the heat production is in some way connected with the reaction of the host to the infecting agent; but so far we have failed to discover the purpose or fate of this excess, and we possess no data which even suggest a plausible hypothesis.

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[42] Five per cent. of the intake has been deducted to cover the loss of calories in the feces.

[43] Magnus-Alsleben, E.: Ueber die Ausscheidung des Kohlenstoffs im Harn., Ztschr. f. klin. Med., 1909, lxviii, 358.

[44] Mohr: Ueber den Stoffzerfall in Fieber, Ztschr. f. klin. Med., 1904, lii, 371.

[45] Müller, F.: Stoffwechseluntersuchungen bei Krebskranken, Ztschr. f. klin. Med., 1889, xvi, 542.

RESPIRATORY QUOTIENTS

Febrile Period.—The averages of the respiratory quotients[46] in Table 15 give a great deal of information as to the different foods actually oxidized by the patients. The fasting individuals in the steep-curve period, with an average quotient of 0.76, were no longer oxidizing carbohydrate in quantity. It is estimated that from 20 to 25 per cent. of the calories were furnished by protein, from 5 to 10 per cent. by carbohydrate and the remainder by body-fat. The high-calory patients in the same period had respiratory quotients of 0.87 and 0.85, indicating that carbohydrate supplied from 40 to 50 per cent. ot the energy. These results demonstrate that carbohydrate is oxidized by the fever patient whenever it is available, and are a farther justification of the clinical procedure of giving large amounts of this foodstuff.

Figure 2 brings out the fact that there is a definite tendency for the respiratory quotient to rise during the last days of the febrile period. We have interpreted this to mean that the reparative processes of convalescence are established before the fever subsides.

Convalescence.—The average respiratory quotient of the high-calory patient rises above 0.92 in the first week of convalescence. The fasting patients did not show a similar rise until the third week, which was probably the time when they began to take large amounts of food. The high average of the quotients is due to the large number above 1, obtained when the regenerating organism is transforming ingested carbohydrate into body-fat. It is extraordinary how long this process may continue after the last meal. Previous observers have repeatedly obtained quotients over 1 from twelve to fourteen hours after the last food, during the period of convalescence. The body apparently makes this transformation with very slight loss of energy, and it is in this manner that the fat depots of the body are in part replenished. It is impossible to say whether or not the body oxidizes any fat during the period when fat is being formed from carbohydrate. If there be any catabolism of this material, it is more than counterbalanced by anabolic processes.

Nineteen quotients over 1 were recorded in the first and second weeks of convalescence in experiments in which there was no question of *Auspumpung*. The highest quotients, 1.1 and 1.11, were found in John H. during convalescence when he was given 28 gm. carbohydrate every hour.

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[46] Nineteen respiratory quotients obtained on patients Frank W. and Louis M. have been omitted from the averages because of the possibility that the height of the quotients was due to *Auspumpung*.

The data concerning the quantitative effect of food on metabolism in individual patients is somewhat fragmentary, and we shall not attempt to formulate conclusions based on the results.

SUMMARY AND CONCLUSIONS

The Benedict "universal respiration apparatus" employed in this investigation was tested on normal controls and found to give accurate results. We believe that the apparatus is well adapted to clinical use if Benedict's technic is carefully followed, that is to say, if precautions are taken to prevent leaks, and if results obtained on patients whose breathing is irregular or labored are discarded. Leaks lower the quotient, irregular and labored breathing artificially raise it.

The metabolism of liberally fed typhoid patients exceeds that of normal individuals by from 36 to 40 per cent. Previous observers have found that the average basal metabolism during the febrile period of patients who are on diets containing less energy than they produce is 35.3 calories per kilogram per day. Comparing this figure with the averages obtained, after ingestion of food, on typhoid patients who received from 35 to 170 calories per kilogram daily, it is observed that the average metabolism per kilogram of body-weight shows an increase of from only 2 to 5 per cent. If the body-surface be made the basis of comparison, it is found that the increase is between 11 and 13 per cent. Since the variation in the metabolism of different normal persons may amount to 10 per cent. above or 10 per cent. below the normal average, it is obvious that the influence of a large ingestion of food cannot be of great importance in increasing heat production in the febrile stage of the disease. In other words, the large amount of food administered exhibits little or no specific dynamic action, thus removing the chief theoretical objection to the use of a liberal diet in typhoid fever.

In the high-calory cases approximately 10 per cent. must be added to cover the increase in metabolism caused by bodily exertion incident to moving about the bed. Accordingly the typhoid patients examined had an average theoretical requirement of 38.5 calories per kilogram per day. It has been found, however, that the theoretical requirement must be exceeded by from 50 to 110 per cent. in order to bring the patient into nitrogen and weight equilibrium. The fate of the excess has not yet been discovered.

In the steep-curve period and during relapses some patients derived from 84 to 89 per cent. of their calories from carbohydrate and, in addition, transformed some carbohydrate into body-fat. The majority of the high-calory patients showed a sharp rise in the level of the respiratory quotients during the last days of the fever, which we have interpreted to mean that the regenerative processes of convalescence begin before the temprature reaches normal.

We desire to express our gratitude to Prof. Graham Lusk for his unfailing interest in our work and for many valuable suggestions during its prosecution. We wish also to thank Dr. Francis G. Benedict, director of the Carnegie Nutrition Laboratory, for helpful advice respecting the handling of the Benedict "universal respiration apparatus" which we employed, and Mr. J. A. Riche for its construction. Our thanks are due to Mr. Frank C. Gephart and Mr. Rudolf H. Harries, of the Russell Sage Institute of Pathology, and to Mr. John M. Janson for aid in food and urine analyses and in the operation of the machine. We are indebted to Dr. Charles L. Dana for the privilege of studying cases in his service at the hospital. For the many food analyses we are indebted to Mr. Frank C. Gephart of the Russell Sage Institute of Pathology.

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A STUDY OF TWENTY-NINE CASES OF ADVANCED PULMONARY TUBERCULOSIS TREATED BY ARTIFICIAL PNEUMOTHORAX *

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The idea that compression of a lung might exert a beneficial effect on pulmonary tuberculosis was first suggested in 1822 by Carson,[1] a physician and physiologist in Liverpool. Ten years later Houghton[2] reported a case of pulmonary tuberculosis in which after the occurrence of spontaneous pneumothorax, improvement in many symptoms took place. During the following fifty years the observation was repeatedly made (Stokes,[3] Spengler,[4] Wellman,[5] Späth,[6] Potain[7] and others) that cases of pulmonary tuberculosis did well when compressed by complicating pleuritic effusions. The resection of ribs resulting in collapse of the lung was advocated by Quincke,[8] Spengler[9] and others and also produced favorable results. The credit for the introduction of the method of compressing a tuberculous lung by means of producing artificial pneumothorax belongs to Forlanini.[10]

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* A preliminary report of this paper was presented before the Internal Section of the New York Academy of Medicine, Oct. 21, 1913.

* From the Bedford Hills Sanatorium of the Montefiore Home.

[1] Carson, James: Essay, Physiological and Practical, Liverpool, 1822.

[2] Houghton: Dublin J. M. and Chem. Sc., 1832, i, 313.

[3] Stokes: Diseases of the Chest, 1837.

[4] Spengler, Lucius: Sur Chirurgie des Pneumothorax, Beitr. z. klin. Chir., 1906, xlix, 69.

[5] Wellman, K.: Erfahrungen in der Behandlung mittels künstlichen Pneumothorax, Beitr. z. Klin. d. Tuberk., 1910, xviii, 81.

[6] Späth: Beziehungen der Lungen-Kompression zur Lungentuberkulose, Med. Correspondez-Blatt des Wurttembergischen ärzt. Landsverein, 1888, lviii, No. 14, p. 105.

[7] Potain: Des injections intro-pleurales d'air stérilisé dans le traitement des épanchements consécutifs au pneumothorax, Gaz. d. Hôp., 1888, lxi, 451.

[8] Quincke, H.: Ueber Pneumotomie bei Phthise, Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1896, i. 234.

[9] Spengler, Lucius: Dauererfolge bei Behandlung schwerer einseitiger Lungentuberkulose mittelst künstlichen Pneumothorax München. med. Wchnschr., 1911, iviii, 460.

[10] Forlanini: Zur Behandlung der Lungenschwindsucht durch künstlich erzeugten Pneumothorax, Deutsch. med. Wchnschr., 1906, xxxii, 1401; Die Indikationen und die Technik des künstlichen Pneumothorax bei der Behandlung der Lungenschwindsucht, Therap. d. Gegenw., 1908, xlix, 485 and 531.

His methods have been adopted by Brauer,[11] Brauer and Spengler,[12] Spengler,[13] Saugmann,[14] Keller,[15] Knoblauch[16] and others, and in this country, chiefly, by Robinson and Floyd[17] and Balboni.[18]

It has been shown that the advantages obtained by the method of artificial pneumothorax are that the lung is immobilized and new connective tissue grows in the atelectatic portions particularly around the bronchi and the vessels (Drasche,[19] Graetz,[20] Mosheim,[21] Warnecke[22] and others). Kaufman[23] has shown that in consequence of prolonged compression of the lung, the connective tissue thus formed causes a marked anatomical and functional injury and that reexpansion of the lung, on withdrawal of compression, does not occur. As further effects of pulmonary compression, tubercles are infiltrated by fibrous tissue, and cavities collapse and become obliterated. Aside from the changes due to the growth of connective tissue, changes in the circulation occur. Stasis in the circulation of the lymph sets in, as

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[11] Brauer, L.: Beobachtungen bei Pneumothorax, Verhandl. d. Cong. f. inn. Med., 1908, xxv, 454, Cong., Vienna. Der therapeutische Pneumothorax, Deutsch. med. Wchnschr., 1906, xxxii, 652; Erfahrungen und Ueberlegungen zur Lungenkollapse. Therapie, Beitr. z. Klin. d. Tuberk., 1909, xii, 49; Ueber Indikationen und therapeutische Erfolge des künstlichen Pneumothorax, Verhandl. d. Deutsch. Gesellsch f. Chir., 1908, xxxvii, 130.

[12] Bräuer, L., and Spengler: Klinische Beobachtungen bei künstlichen Pneumothorax, Beitr. z. Klin. d. Tuberk., 1911, xix, 1.

[13] Spengler, L.: Ueber mehrere Falle von geheilten tuberkulosen Pneumothorax mit gleichzeitiger Heilung der Tuberkulose. Ztschr. f. Tuberk. u. Heilstättenw., 1901, ii, Nos. 1 and 2; Ueber Lungenkollapsetherapie, Cor.-Bl. f. Schweiz. Aerzt., 1913, xliii, 1025-1039.

[14] Saugmann, C., quoted by von Muralt: Beitr. z. Klin. d. Tuberk., 1911, xviii, 373; Ueber die Anwendung des künstlichen Pneumothorax in der Behandlung der Lungentuberkulose, Ztschr. f. Tuberk., 1908, xxi, 1.

[15] Keller: Erfahrungen über den künstlichen Pneumothorax, Beitr. z. Klin. d. Tuberk., 1912, xxii, 165.

[16] Knoblauch, quoted by Bräuer and Spengler: Beitr. z. Klin. d. Tuberk., 1912, xxii, 165.

[17] Robinson, Samuel and Floyd, Cleveland: Artificial Pneumothorax as a Treatment of Pulmonary Tuberculosis, The Archives Int. Med., April 15, 1912, p. 452.

[18] Balboni, Gerardo M.: The Treatment of Pulmonary Tuberculosis by Artificial Pneumothorax According to the Method of Forlanini, Med. and Surg. Jour., Boston, 1912, clxvii, 755, 804, 844, 882, 928.

[19] Drasche: Ueber die operative Behandlung des tuberkulosen Pneumothorax. Wein. klin. Wchnschr., 1899, xii, 1117 and 1149.

[20] Graetz, F.: Der Einfluss der künstlichen Pneumothorax auf die tuberkulose Lunge, Beitr. z. Klin. d. Tuberk., 1908, x, 249.

[21] Mosheim, B.: Die Heilungsaussichten der Lungentuberkulose bei Spontanen und künstlichen Pneumothorax, Beitr. z. Klin. d. Tuberk., 1904-5, iii, 331-396.

[22] Warnecke: Beitrag zum pathologischen Anatomie des künstlichen Pneumothorax, Beitr. z. Klin. d. Tuberk., 1910, xvi, 171.

[23] Kaufmann, K.: Ueber die Veränderungen der Pleura und Lunge gesunder Hunde durch kunstlichen Pneumothorax, Beitr. z. klin. d. Tuberk., 1912, xxiii, 57.

Molhuysen[24] has shown in his experiments, and this favors a diminution in the degree of toxemia. The total amount of blood in the lungs is reported to diminish (O. Bruns[25]) but the experimental evidence for this finding is unsatisfactory.

The number of cases treated by this method is still few when the advantages claimed for the treatment are considered, and the precise observations of the technic, the clinical history and the mechanism of action are sufficiently incomplete to induce us to publish our investigations. The twenty-nine cases (thirteen male and sixteen female) which form the basis of this report were studied during the six months from April to October, 1913. The patients were all in the third stage of the disease.[26] In this series of cases the frequency with which the greater involvement occurred on the left side is striking; the left side was involved in twenty and the right in nine cases.

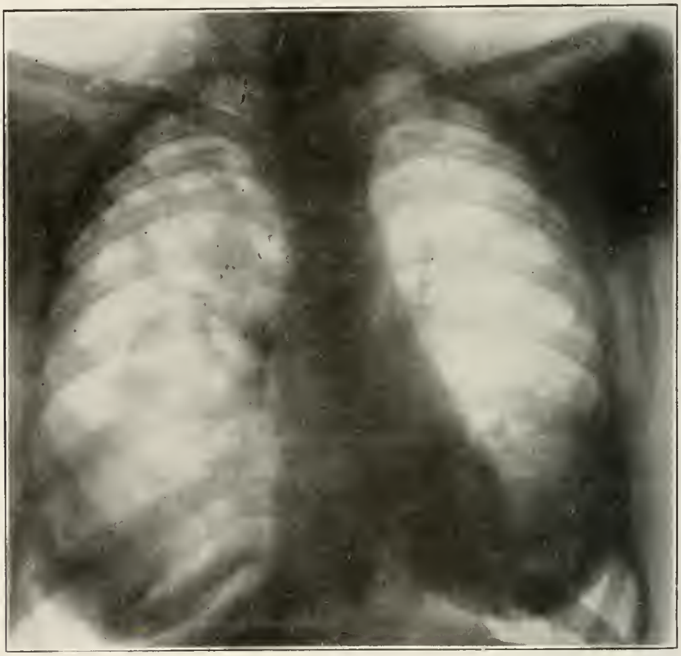

Fig. 1.—M. R. Bilateral tuberculosis four years standing. Actively progressive on right side. Sanitarium care for two and a half years. Progressive. Partial right-sided pneumothorax. Extensive adhesions prevent collapse. Slight relief of symptoms.

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[24] Molhuysen: Dissertation, Leyden, 1911; quoted by Von den Bergh.

[25] Bruns, O.: Ueber Folgezustande des einseitigen Pneumothorax. Experimentelle Studien, Beitr. z. Klin. d. Tuberk., 1908, xiii.

[26] In this paper the classification adopted by the National Association for the Study and Prevention of Tuberculosis has been used.

In our procedure we gave the patients morphin (1/8 to 1/4 grain) and atropin (1/150 grain hypodermatically) ten minutes before operation. The skin was sterilized with tincture of iodin and the excess was removed with 95 per cent. alcohol. The area was anesthetized with 1 per cent. novocain and 1 or 2 c.c. of 1: 10,000 epinephrin (adrenalin, P. D. & Co.).

The apparatus introduced by Floyd and Robinson,[17] and Floyd's needles, were used. A large-caliber needle was employed for the initial inflation and a smaller one in subsequent treatments. The patient was placed on his side on a pillow in order to widen the intercostal spaces. The skin was punctured, and not incised, with a fine cataract knife protected with adhesive plaster, to prevent injury and to avoid bleeding. As the needle carrying a stilet was slowly introduced through the anesthetized area, perpendicularly to the skin, into the pleural cavity, the operator experienced a sense as of withdrawal of resistance. This sensation is quite characteristic. A water manometer was attached to the needle and when this was in place several readings were taken. When these became constant and the respirations became regular and when the oscillations in the manometer were free and the intrapleural pressure negative, nitrogen gas was permitted to flow. The rate was approximately 50 c.c. per minute. The intrapleural pressure was read after each 100 c.c. had entered. The total amount introduced was from 700 to 800 c.c., in our early cases, but later we reduced it to 300 to 500 c.c. and obtained better results To check severe pulmonary hemorrhage we believe large initial amounts are indicated (Cases 21 and 28). We calculated that an expansion of about 10 per cent. took place in the volume of gas introduced. When the needle was withdrawn, the index-finger of the opposite hand was pressed firmly over the site of the puncture and a pressure bandage was applied. This procedure prevented the occurrence of subcutaneous emphysema, which had been troublesome before we resorted to this precaution. In withdrawing the needle it is advisable to measure and note, as a guide for subsequent treatment, the depth to which it had been introduced in order to enter the pleural cavity.

The area to select as the site of injection is the one most likely to be free from adhesions. The axilla was preferred because there the intercostal spaces are wide and there are fewer layers of muscle. The places to avoid are those over cavities, the precordium, the region of the large vessels, the breast tissue in women and the region posteriorly below the ninth space. We have frequently been obliged to puncture posteriorly and found the dense layers of muscle and the narrow intercostal spaces distinct disadvantages.

The diagnosis and localization of adhesions is one of the most important subjects to be considered when the treatment of artificial pneumothorax is undertaken, and we wish to give it special emphasis. This subject has been studied by Kistler,[27] Hamman and Sloan,[28] King,[29] Otis,[30] Konselmann,[31] Lapham,[32] Lillingston,[33] Rhodes,[34] Montgomery,[35] Klemperer,[36] Schmidt[37] and others. It depends on a careful consideration of the history, attacks of pleurisy for example, the physical examination and the fluoroscopic picture. The absence of a history of pain is no indication that the pleural cavity is free from adhesions. The methods used to determine whether a pleural space is free from adhesions, namely, physical diagnosis, fluoroscopy and roentgenoscopy, although important, are also often of uncertain value. Adhesions, for example, are usually denser than can be demonstrated by a roentgenogram (Necropsy report Case 4 and Fig. 4). Roentgenograms of the chest of each patient were taken before treatment began and every patient was fluoroscoped within twenty-four hours after each treatment. Roentgenograms were also taken at intervals during treatment. We wish to emphasize the fact that the extent of disease, the completeness of the pneumothorax and adhesions preventing complete collapse, were better demonstrated by the plate than by the fluoroscope (Figs. 1 and 6). The fluoroscope, on the other hand, showed better small amounts of gas or fluid and angulations of the diaphragm (Case 18). To detect small effusions it is advisable,

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27. Kistler, E.: Beitrag zur pathologischen Anatomie des. künstlichen Pneumothorax, Beitr. z. Klin. der Tuberk., 1911, xix, 459.

28. Hamman and Sloan: Induced Pneumothorax in Treatment of Pulmonary Disease, Johns Hopkins Hosp. Bull., 1913, xxiv, 53.

29. King, Herbert M., and Mills, Charles: Therapeutic Artificial Pneumothorax, Am. Jour. Med. Sc., 1913, cxlvi, 330.

30. Otis, E. G.: Artificial Pneumothorax in Advanced Unilateral Cases of Pulmonary Tuberculosis, Med. and Surg. Jour., Boston, 1912, clxvii, 612.

31. Konselmann: Ueber den Einfluss pleuritischer Exudate auf den Verlauf der Lungentuberkulose, Beitr. z. Klin. d. Tuberk., 1908, x, 373.

32. Lapham, Mary: Eighth Annual Report of National Association for the Study and Prevention of Tuberculosis, Washington, 1912, p. 371; The Treatment of Pulmonary Tuberculosis by Compression of the Lung, Am. Jour. Med. Sc., 1912, cxliii, 503.

33. Lillingston, C.: Treatment of Pulmonary Tuberculosis by Artificial Pneumothorax, Lancet, London, 1912, 1669.

34. Rhodes, Herbert: The Treatment of Pulmonary Tuberculosis by Inducing Artificial Pneumothorax, Brit. Med. Jour., 1911, ii, 1062.

35. Montgomery, Charles M.: Pleural Effusion Due to Artificial Pneumothorax, Jour. Am. Med. Assn., Feb. 15, 1913, p. 494.

36. Klemperer, F.: Zur Behandlung der Lungenphthise mit künstlichen Pneumothorax, Therap. d. Gegenw., 1906, xlvii, 545.

37. Schmidt, Adolf: Erfahrungen mit dem therapeutischen Pneumo- und Hydrothorax bei einseitiger Lungentuberkulose, Bronchiektasien und Aspirations Erkrankungen, Beitr. z. Klin. d. Tuberk., 1908, ix, 261.

when making a roentgenogram, to place the patient in an upright position. After all care is taken, the results of inflation differ from one's expectations. For instance, in cases in which we expected to encounter difficulties we often found none (Cases 1, 4, 9, 12), whereas in other cases in which we suspected no extensive pleuritic involvement, there were adhesions which rendered the treatment partially or totally impossible (Cases 15, 17, 24 and 25). This occurred in about 15 per cent. of our patients. The reports embracing a large series of cases (Saugmann,[14] Brauer and Spengler[12]) show from 20 to 30 per cent. failures. Complete collapse of the lung is obtainable in spite of the presence of adhesions (Figs. 3 and 6). In three of our patients (Cases 14, 17 and 24) initial negative pressures were rapidly neutralized by the introduction of small amounts of gas. The subsequent readings were all zero with fairly wide oscillations, negative and positive. This phenomenon was repeatedly observed and not even after the introduction of large amounts (Case 17, 1,800 c.c.) of nitrogen were we able to detect its presence in the pleura by either physical

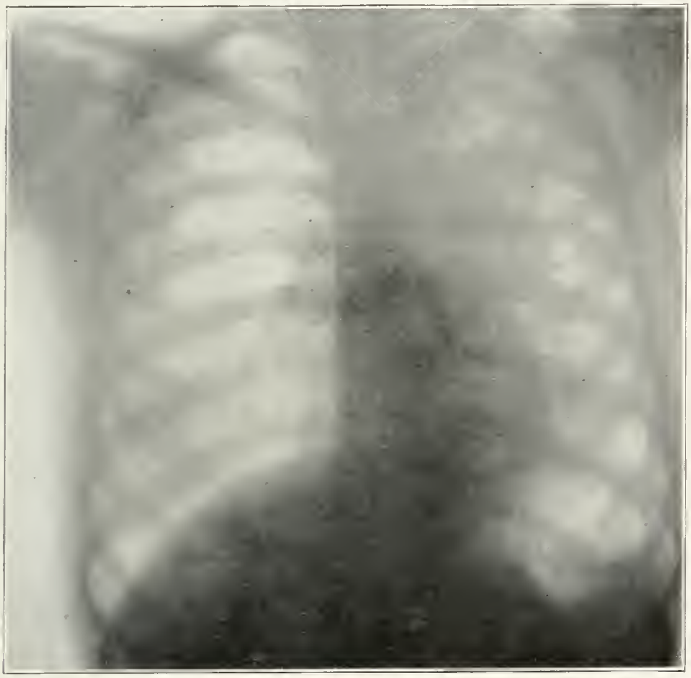

Fig. 2.—R. G. Photograph taken before induction of artificial pneumothorax. Shows extensive tuberculosis of left lung and several small cavities in upper lobe; miliary infiltration in right upper lobe. Clinically—Actively progressive tuberculosis on left side; slight at right apex.

examinations or fluoroscopy. In these cases the gas was probably introduced directly into the lung.

It is the use of the manometer, first introduced by C. Saugmann[14] in 1907 and modified by von Muralt,[38] which has rendered the creation of a pneumothorax safe and easy. It yields definite indications of the presence of adhesions. It gives reliable information as to where the needle-point is situated, recording wide oscillations with negative pressure only in a free pleural space. We have found that very short negative-pressure oscillations, becoming rapidly positive on the introduction of small amounts of gas, indicate the presence of a pleural pocket and that the amount of gas required to overcome the negative pressure bears a direct relation to the size of the pocket. In the presence of adhesions a positive reading becomes less positive or even negative when the adhesions are broken down. When the initial oscillations fluctuate about zero one suspects that the needle-point is

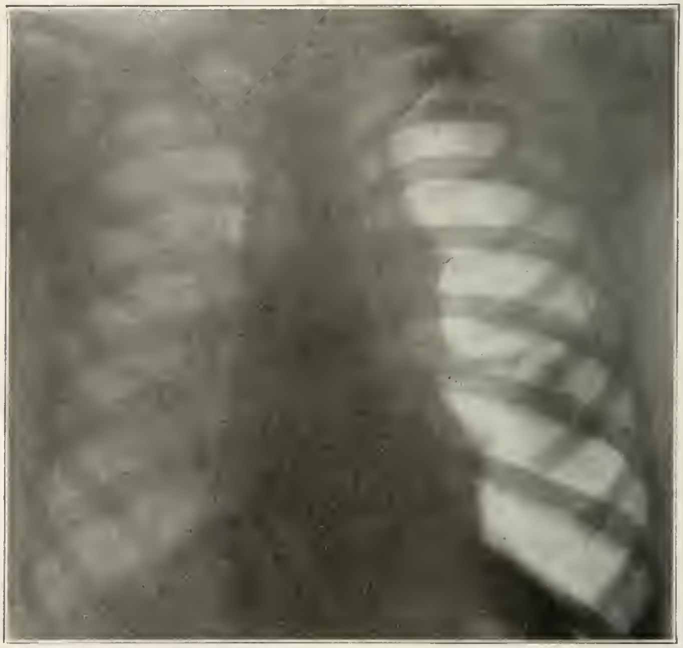

Fig. 3.—R. G. Advanced tuberculosis of left upper lobe with cavity formation; slight tuberculous infiltration at right apex. Photograph taken six weeks after induction of pneumothorax. Almost complete collapse of left lung. Broad band of adhesion from visceral pleura to seventh rib. Amelioration of all symptoms. Patient illustrates case suitable for treatment.

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[38] Von Muralt, L.: Manometrische Beobachtungen bei der Ausübung der Therapie des künstlichen Pneumothorax, Beitr. z. Klin. d. Tuberk., 1911, xviii, 359.

in the pulmonary parenchyma. An initial slightly positive rise uninfluenced by respiration is said to occur when the needle enters a blood-vessel. An initial negative pressure increasing in degree with every successive inspiration, but remaining unchanged by expiration, occurs, we believe, when the needle-point lies immediately outside the parietal pleura. The treatment should be terminated when the pressure becomes slightly positive, for should the mediastinum be diseased, too great pressure might cause its rupture (Case 12, Fig. 5). To raise the pressure unduly in order to overcome the resistance of adhesions may be followed by dangerous consequences, such as tearing the lung, hemorrhage, air-embolism, infection and extension of the tuberculous process.

When pain is experienced during the introduction of gas, it is commonly due to stretching of adhesions. It is usually of short duration and its severity is diminished at each succeeding treatment. In several of our satisfactorily established pneumothoraces, indeed, the presence of numerous adhesions caused no discomfort. When pain and hyperalgesia are referred to the region of the shoulder on the side treated (Case 18, Fig. 7) they form a diagnostic point of much interest. Mackenzie[39] has called attention to the fact that, when the diaphragmatic pleura is irritated, stimuli ascend from it by the phrenic nerves to the nuclei of the fourth and fifth cervical nerves. Nerves from these centers are distributed to the shoulders and it is to these that the pain is referred. He observed the phenomenon in cases of diaphragmatic pleurisy.

When complete collapse of the lung is established the physical signs undergo great changes. On the untreated side exaggerated and harsh breathing is heard, but we have failed to notice the disappearance of signs of consolidation on the untreated side mentioned by several observers (Brauer,[11] Forlanini[10] and others). In some of our patients (Cases 11, 13, 18) we observed an extension of the physical signs on the untreated side following a period of apparent improvement. A reasonable explanation for the spread of disease on the untreated side is that offered by Van den Bergh, de Jong and Shüt.[40] From a study of their necropsy findings and from the experimental work of Tendeloo they noted that the greatest expansion in the untreated lung occurred at the periphery and basic portions. These, on account of their greater respiratory volume change, as Tendeloo[41] showed, suck the lymph from the less mobile portion and so draw infected particles to the healthy tissue. On the treated side râles become less marked, or are absent; the signs of cavity disappear. To determine the presence of complete collapse, physical signs alone may be misleading, for many factors influence the breath sounds in pneumothorax. The respiratory effort of the patient, the capacity for sound transmission inherent in the intervening medium and the resonating qualities of the thoracic chamber, tend to modify them. A ringing, or metallic quality, of the whispered voice we believe to be the most characteristic physical sign of well-established compression. To determine the degree of compression we have found fluoroscopy and roentgenography more reliable than physical examination.

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[39] Mackenzie, J.: Symptoms and Their Interpretation, 1909, p. 209.

[40] Hymans vander Bergh, de Josse Lin de Jong, Shut H.: Einige Erfahrungen mit künstlichen Pneumothorax, Beitr. z. klin. d. Tuberk., 1913, xxvi, 47, No. 1.

[41] Tendeloo; quoted by Van der Bergh, de Jong and Schüt (footnote 40).

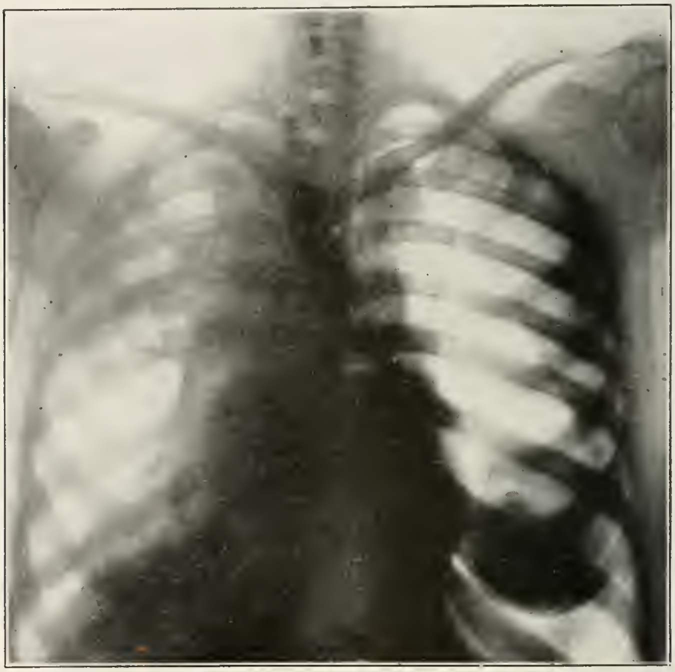

Fig. 4.—Y. N. Extensive bilateral tuberculosis. Pneumothorax induced for relief of symptoms. Complete compression of left lung and band of adhesion between visceral pleura and second left interspace. Autopsy—Left lung and histologic sections shown. Chronic endocarditis.

Many complications have been reported to occur during the production of an artificial pneumothorax. We enumerate a few:

1. Pleural reflex, shock, or eclampsia, occurred in one of our cases (Case 11) after the needle had been introduced but before the gas had been permitted to enter the chest. This phenomenon has been regarded by some, particularly in the German clinics, as due to gas

emboli, but the time of its occurrence in this instance seems to preclude this possibility. In the three necropsies reported by Sundberg,[42] no evidence of emboli was found. In one of these, death occurred before the gas had been turned on.

2. Pain from a possible injury of an intercostal nerve, or one of its branches, is a complication which we observed in three cases (Cases 9, 11 and 18) after several inflations had been made. It was severe and there was exquisite tenderness in the lower left axilla between the seventh and ninth spaces.

3. Subcutaneous emphysema may be caused by the escape of gas before the needle has entered the pleural cavity. It occurs also during violent coughing, or from leakage of gas along the side of the needle. We encountered this complication in a few of our cases.

4. Of deep emphysema, we have no personal experience.

5. We have seen blood-stained expectoration in a few instances caused by the puncture of the lung by the needle. This disappeared promptly.

6. Rupture of the mediastinum with bilateral pneumothorax was observed once (Case 12, Fig. 5), but no untoward results ensued.

7. Of embolism we have no personal experience; it is probably easy to avoid by careful technic.

8. Attacks of vomiting were observed once[43] (Case 1) in our series. The right lung in this patient had been compressed.

9. A small amount of pleural effusion was observed in seven cases (Cases 11, 13, 18, 21, 23, 24, 29). This complication was always accompanied by a sudden rise of temperature to 102 or 103 F., and by pain in the side. These two symptoms are characteristic. The fluid aspirated from these cases was turbid, either straw-colored or serosanguineous. It showed a large number of lymphocytes. The fluid from five of these was inoculated into guinea-pigs and they developed tuberculosis. We are uncertain concerning the mechanism of production of this type of pleurisy and we hope to report in greater detail on this subject at a later date. Effusions are reported to occur in about one-third of the treated cases. Succussion and a tilting of the level of the fluid seen with the fluoroscope, on changing the position of the patient, have been the two most constant signs of pleural effusion in our series.

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[42] Sundberg, C.: Drei Todesfälle mit Obduktion Behandlung von Lungentuberkulose mit künstlichen Pneumothorax, Beitr. z. Klin. d. Tuberk., 1913, xxvi, 503.

[43] In a case (right-sided) observed by one of us, vomiting was noted several times promptly following the introduction of gas.

Certain contraindications to the treatment should be mentioned. They include:

1. The presence of the first and second stages of the disease in patients who are doing well under hygienic and dietetic treatment.

2. Marked mental apprehension.

3. Chronic heart-disease, or marked chronic nephritis.

4. Definite intestinal tuberculosis.[44]

The indications for the treatment may be summarized as follows:

1. Extensive unilateral progressive lesions which have failed to improve under hygienic and dietetic treatment. The earlier it is recognized that sanatorium treatment is useless, the better the chance of succeeding with pneumothorax treatment.

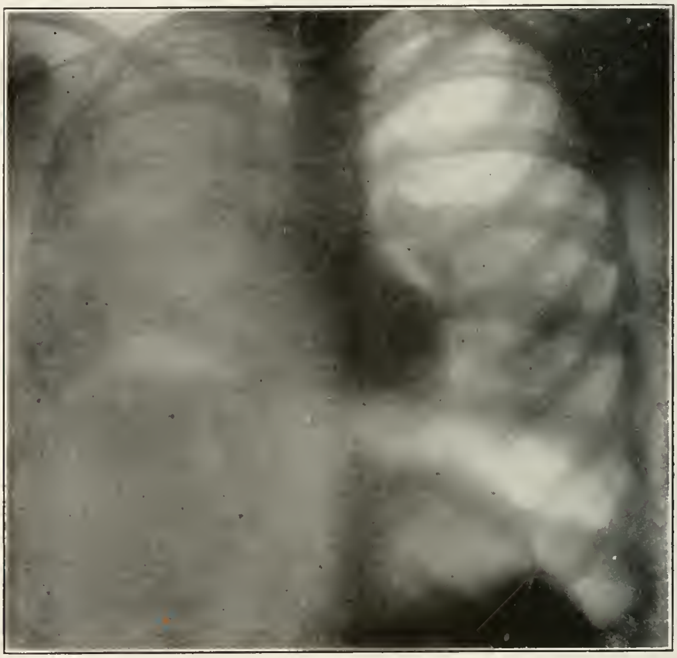

Fig. 5.—A. P. Extensive tuberculosis of right lung. Massive tuberculous consolidation at base. Small clear area at right base, larger one at left base. Pneumothorax induced on right side; rupture through mediastinum.

2. Extensive chronic unilateral lesions which remain stationary and show no constitutional symptoms, but which cause distressing cough and excessive expectoration.

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[44] In a patient observed by one of us, a tentative diagnosis of intestinal tuberculosis had been made two years ago owing to a distressing diarrhea alternating with constipation. This symptom promptly disappeared with the establishment of a therapeutic pneumothorax.

3. Involvement of a single lobe containing a cavity.

4. Extensive unilateral progressive lesions with small inactive involvement of the opposite side.

5. Bilateral involvement with extensive lesions, slowly progressive on one side and little, or no, activity on the other.

6. Repeated hemoptyses which do not cease under the usual treatment (Cases 17, 21, 28); also, less frequently, large pulmonary hemorrhages when the loss of blood is great enough to warrant a fatal prognosis (Rothschild[45]).

7. Advanced progressive tuberculosis suggesting an early fatal issue. This refers to a group of cases to which we wish to call especial attention. Under ordinary circumstances the induction of an artificial pneumothorax is here contraindicated; but a certain number of patients by proper care and selection are found, who, when they are treated, afford examples of gratifying relief from very distressing symptoms where none has otherwise been possible. We have treated several such patients with results that have encouraged us to recommend an extension of the indications for the treatment, when this can be accomplished in a careful and conservative fashion.[46]

The clinical history of patients treated by artificial pneumothorax, when favorably influenced, shows a decrease in the temperature either abrupt or gradual, depending on the extent and character of the process and on the degree of compression (Cases 3 and 19 with temperature charts and Figs. 2, 3 and 8). With the drop in temperature there is usually slowing of the pulse-rate, disappearance of night-sweats, improved appetite and digestion and a feeling of general well-being. The cough and expectoration in many cases are at first aggravated but usually at the end of two or three days these symptoms decrease in severity and disappear. Diminished cough and expectoration permit sleep which, in spite of the use of hypnotics, has often been absent for months. It is noteworthy that no gain in weight commensurate with the amelioration of symptoms, takes place (Cases 2, 19, 20, 21, 22, 23, 27, 28, 29). Many patients who were relieved of their symptoms have an initial loss of weight which in some has been progressive (Cases 1, 19, 28). Even the cases in which we have succeeded best have shown an initial loss which persisted for several months before a gain was noticed (Cases 2 and 3). We are unable to explain this result. That it is not dependent on anorexia we are certain, for the appetite of many patients improved from the beginning of treatment. Blood-pressure readings taken before, during and after treatment failed to show constant changes.

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[45] Rothschild: The Treatment of Hemoptysis in Pulmonary Tuberculosis, Jour. Am. Med. Assn., Oct. 28, 1911, p. 1424.

[46] There is a group of patients, not included in this series, in whom we believe this form of treatment may be applicable. We refer to those patients who progress fairly rapidly from Stage I to Stage II, with evidence of marked toxemia and who under ordinary circumstances pursue a speedily fatal course.

A striking feature which indicates improvement is a change in the mental attitude. Patients who had been bed-ridden for many months wished to be up and about (Cases 2, 3, 4, 7, 10, 12, 19, 20, 21, 22, 23, 27, 28, 29); some took moderate exercise, others were able to dance and play ball and a few made excursions to the city, a distance of 40 miles, without ill effects.

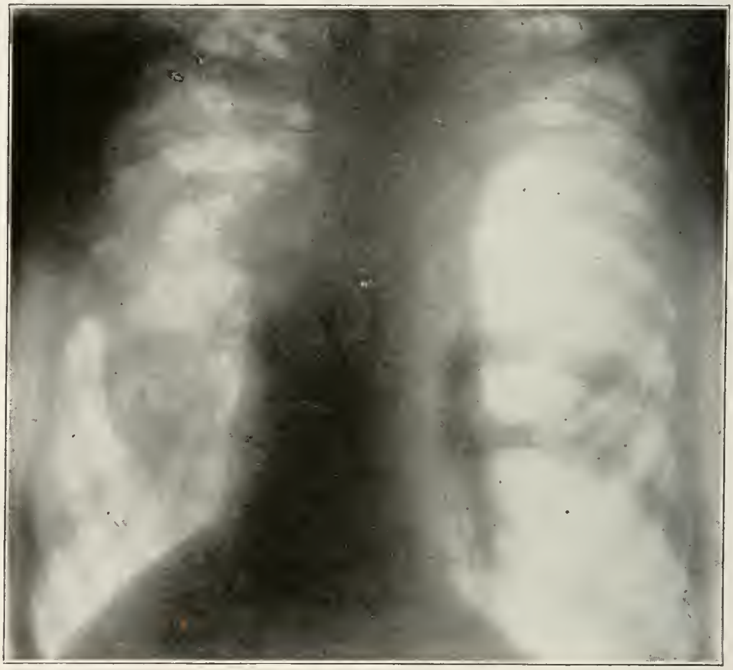

Fig. 6.—M. K. Extensive bilateral tuberculosis. Complete collapse of left lung. Band of adhesion seen just below clavicle. Induced for relief of symptoms.

Numerous observers have reported a marked diminution and in some cases a disappearance, of tubercle bacilli from the sputum. We have not noticed any appreciable change in their number. The cases most benefited were usually those in which compression was most readily and completely accomplished, but clinical improvement sometimes followed in cases in which only a moderate degree of compression was possible (Cases 12 and 23). The ultimate aim of this plan of treatment, the purpose of which is to promote fibrosis, is best secured by causing complete collapse of the lung by the introduction of moderate amounts of gas frequently repeated, rather than with larger amounts at greater intervals. The most desirable condition is that in which a slightly positive intrathoracic pressure is maintained at all times.

The indications which warrant reinflation are:

1. Persistent evidences of toxemia, such as fever.
2. A return of troublesome cough and expectoration.
3. Evidences of incomplete compression of the lung. The more complete the compression the longer the interval between inflations.

The contraindications to reinflation are:

1. Marked pain.
2. Urgent dyspnea.
3. Subcutaneous emphysema.
4. Extension of disease on the opposite side.
5. Large pleural effusions.

A report on the results of twenty-nine cases of tuberculosis treated by artificial pneumothorax is offered.

1. A study of the pleural adhesions in each case is important. Roentgenography and fluoroscopy are valuable guides in the study of these cases. The manometer during the induction of an artificial pneumothorax is indispensable.
2. The operation is practically devoid of danger if careful technic is observed.
3. A consideration of the physical signs alone yields insufficient data to form the basis of treatment.
4. In all progressive cases of pulmonary tuberculosis the induction of an artificial pneumothorax should be considered.
5. In some "hopeless" cases it has been useful for it has brought remarkable relief from distressing symptoms, not obtained by any other measure.

We wish to take this opportunity to express thanks to the superintendent and house staff at Bedford, and to Mr. Theodore Scholtz, the radiographist, for their willing aid in carrying out this work.

REPORTS OF CASES

Case 1.—Woman, aged 26, Russian, single, embroidery-maker, admitted Aug. 21, 1911 (Stage III). She has been troubled with cough and expectoration for the past four years. She spent two and a half years in a sanatorium and has had a course of tuberculin treatment. The tuberculous process involved the entire right lung, in the upper lobe of which there was a large cavity (Stage III), and also the left upper lobe which was infiltrated (Stage II). The disease was progressive. She suffered from frequent attacks of pyrexia, excessive cough and expectoration and persistent loss of weight.

The first inflation of nitrogen was given in April, 1913. Since then fourteen inflations have been given with no marked change in the patient's condition. Figure 1 shows to what extent pleural adhesions prevented complete collapse of the lung. Dyspnea has been a marked feature since the beginning of treatment and we are inclined to attribute it to the introduction of too large amounts of gas in the early inflations. Mediastinal and pulmonary adhesions probably allowed only a little displacement of the heart. The expectoration has been diminished; other symptoms remain the same as before the establishment of the pneumothorax.

Case 2.—Woman, aged 35, Hungarian, married, housewife, admitted July, 1912. She gave a three years' history of profuse cough and expectoration, occasional attacks of fever and night-sweats. While in the sanatorium her temperature, for six months before the establishment of artificial pneumothorax ranged, daily from 99 to 104 F. Her appetite was very poor and her weakness progressive. She presented extensive involvement of the right upper and middle lobes (Stage III). A cavity extended from the first to the third intercostal space. An old inactive process was present in the left upper lobe (Stage I).

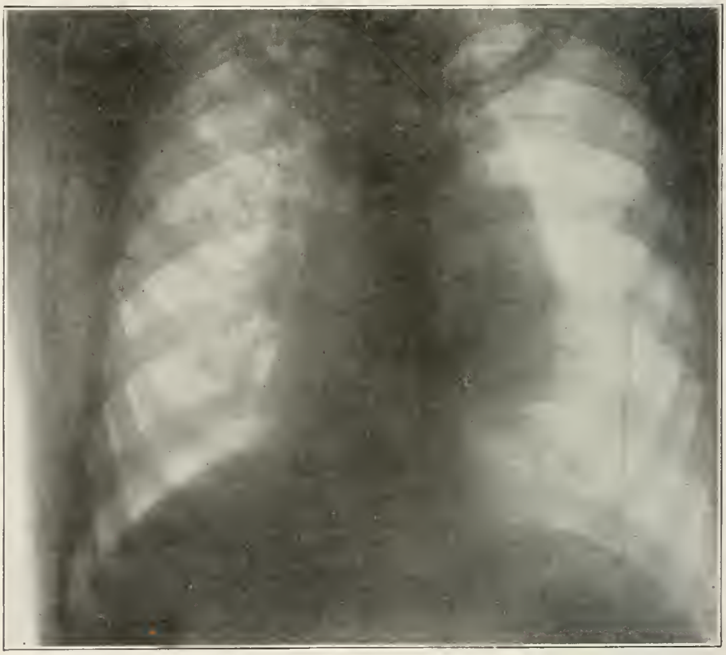

Fig. 7.—M. H. Photograph showing artificial pneumothorax of left side, moderate degree of collapse of left upper lobe with very little compression of left lower. Adhesions between visceral pleura and diaphragm. Hyperalgesia of left shoulder region.

The first nitrogen inflation was given January, 1913, and compression of the lung has been maintained ever since. An immediate improvement occurred. The temperature quickly dropped to normal, the cough and expectoration were markedly reduced and her appetite returned. Her menses have reappeared after an absence of fifteen months. The loss of weight before inflation had been progressive; for four months after beginning treatment her weight remained stationary, or fluctuated slightly. For the past four months she has made a steady gain in weight. The improvement has been progressive. There has been no evidence of activity on the untreated side.

Case 3.—Woman, aged 20, single, Hungarian, stenographer, admitted July 16, 1913. (Stage III.) Her history was one of acute progressive illness for the past six months, with cough, hemoptyses, loss of weight, weakness and pains in the chest. On admission her temperature ranged between 97 and 103 F. She showed an extensive tuberculosis of the left lung. There were several small cavities in the left upper lobe (Stage III). The roentgenogram showed miliary infiltration in the right upper lobe (Stage I). The toxemia was marked.

July 30, 1913, 500 c.c. of nitrogen gas were injected into her left pleural cavity. In the following two weeks she received three more inflations which produced compression of her left lung. Her temperature was brought to normal and has remained there since. Her pulse-rate was reduced from between 120 and 130 to 86. Her cough has been markedly influenced and the amount of expectoration has fallen from 200 gm. daily to less than 30 gm. During the past two months there has been a steady gain in weight. The patient has been out of bed for some time and the influence on her spirits has been striking. Figures 2 and 3 show the pulmonary condition before and after the establishment of a pneumothorax.

Case 4.—Woman, aged 32, married, Hungarian, housewife, admitted April 7, 1913. (Stage III.) A hemoptysis occurring two and a half years ago was the first evidence of pulmonary disease. She was troubled with dry cough for some months thereafter. This was followed by cough with gradually increasing expectoration, dyspnea, anorexia, attacks of chills, fever and sweats. Recently she has been having three or more loose movements daily; her weakness has been progressive. She presented extensive tuberculosis of the entire left side with a cavity in the left upper lobe. (Stage III.) There was also well-marked disease of her right lung (Stage II), and a compensated mitral insufficiency. Her temperature on admission ranged between 100 and 104 F. This temperature continued; the disease was progressive and the weakness and emaciation marked. She has considerable dyspnea and moderate cyanosis. Her most troublesome symptom was her cough which we were unable to control and which prevented sleep. She had observed the beneficial influence exerted on other patients by the establishment of an artificial pneumothorax and begged us to try the treatment in her case. She presented so many contraindications that we were loth to attempt it. Finally, however, in the hope of relieving the intractable cough, we induced a left-sided pneumothorax. For ten weeks following the compression of the lung the benefit was very marked with the exception of the dyspnea, which was increased. All of the other symptoms were greatly relieved. The cough was sufficiently relieved to render sleep possible. The temperature remained below 100 F. and the patient was able to leave her bed.

Three months after the establishment of the artificial pneumothorax she began to do badly. Her temperature rose and her heart became decompensated. In spite of an attempt to withdraw the nitrogen from her chest she died.

At necropsy, performed by Dr. Felberbaum, the pathologist of the Montefiore Home, the right pleural cavity was found obliterated by adhesions. The left pleural cavity contained 3 ounces of fluid and was free, with the exception of two dense bands of adhesions which extended from the visceral pleura to the second and fourth intercostal spaces respectively. The left lung was firmly compressed against the spinal column and the posterior mediastinum. The pleura was very much thickened; the pericardium was adherent to the mediastinal surface of the left lung. An incision into the upper and lower lobes revealed extensive tuberculous bronchopneumonia. Sections taken from this lung showed an extensive healing process; the tubercles were well encapsulated and in many places there was a rich growth of young connective-tissue cells replacing the tubercles. The granulation tissue in the atelectatic area was very extensive. The accompanying microphotographs have been made from these sections (Figs. 9 and 10). The heart showed chronic endocarditis. There was some dilatation of the right ventricle. The ileocecal junction was the seat of a tubercular process.

Case 5.—Woman, aged 20, single, Turkish, operator, admitted Jan. 22, 1913. Stage III. She presented extensive disease of the left lung (Stage III) and slight infiltration at the right apex (Stage I). An artificial pneumothorax was established in this case because of repeated hemoptyses accompanied by rises in temperature. Since the institution of treatment there have been no hemoptyses.

Case 6.—Woman, aged 20, single, Austrian, waist-maker, admitted Nov. 6, 1912 (Stage III). She has had a history of cough and expectoration since June, 1911. She presented signs of extensive involvement of the left lung (Stage III). There were slight changes at the right apex (Stage I). The roentgenogram revealed a cavity on the left side between the third and sixth dorsal vertebrae. The physical signs had grown steadily more extensive; there were occasional rises of temperature. The loss of weight had been progressive since March, 1913. July 2, 1913, an artificial pneumothorax was performed.

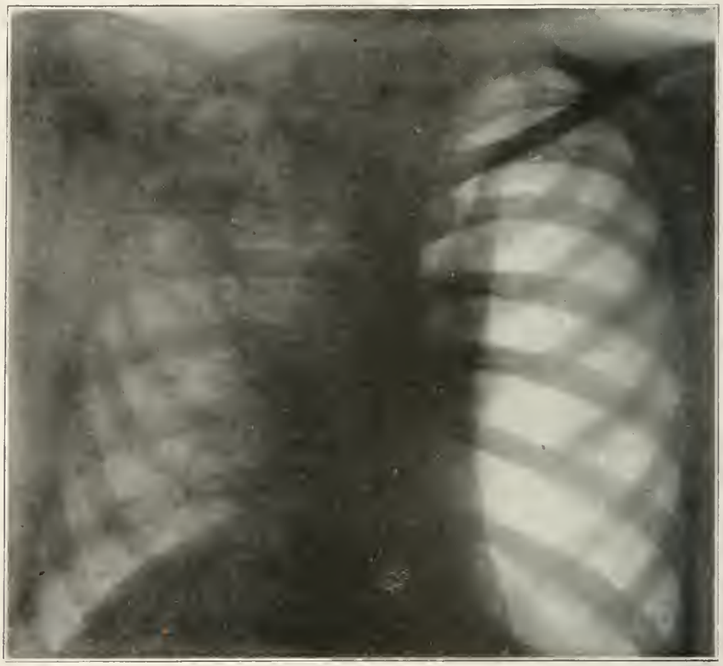

Fig. 8.—J. W. Extensive bilateral tuberculosis. Left pneumothorax. Complete collapse of lung. Amelioration of all symptoms.

Examination.—Sept. 4, 1913: the left lung has been well compressed, the cough and expectoration diminished, the temperature has remained normal, the loss in weight has ceased and the general condition is good.

Case 7.—Woman, aged 21, single, Austrian, operator, admitted April 4, 1913. (Stage III). The patient has had cough, expectoration and thoracic pain for seven months. She presented extensive infiltration of right lung with a cavity two inches in diameter in the right upper lobe at the level of the first rib

(Stage III). There was dulness, increased breathing and dry râles over the left upper lobe (Stage II). The progressive character of her disease and the persistent loss of weight and strength induced us to attempt to compress her right lung. Three weeks after the primary inflation a satisfactory compression had been established. Her cough and expectoration have been diminished. The patient is looking and feeling better. Her appetite has been materially improved. The persistent loss of weight has been checked.

Case 8.—Woman, aged 30, married, Russian, housewife, admitted Sept. 16, 1912. On admission the patient presented a moderately active process in her left upper lobe (Stage III). In spite of eight months' sanatorium care her disease was slowly progressive. She had fairly constant temperature between 100 and 101 F. The roentgenogram later showed the presence of two small cavities in the left upper lobe. The unilateral progressive character of the disease presented the indication for pulmonary compression.

Examination.—Sept. 10, 1913: patient has had fourteen inflations and an excellent compression has been maintained. Her temperature is normal. Her general condition is fair and improved and the loss of weight which had been progressive during the latter part of the spring has been checked. During the past two weeks she has gained two pounds. Her cough has been a troublesome symptom, both before and since the establishment of the pneumothorax.

Sept. 24, 1913: there has been no evidence of activity on the untreated side. Cauterization of a lingual tonsil has checked the cough.

Case 9.—Woman, aged 30, married, Russian, housewife, admitted June 2, 1912 (Stage III). The patient stated that her illness dated back only four months and that it had followed an attack of influenza. Her condition on admission was very poor. She showed general infiltration of the left lung with a cavity in the lower lobe (Stage III); the right apex was infiltrated, but no râles were heard (Stage I). The temperature ranged between 100 and 103 F., and the cough and expectoration were excessive. June 18 the first inflation with nitrogen was given and a pneumothorax was rapidly established. July 9 an hemoptysis of 13 ounces occurred. This so terrified the patient that for ten days she absolutely refused to allow us to continue with her pulmonary compression. During this period she had daily hemoptyses. July 19 treatment was resumed. Gas was introduced on this day and again on July 22. The hemoptyses ceased promptly. With the establishment of a complete pneumothorax the general condition improved; the cough and expectoration were markedly reduced and the temperature remained practically normal. The loss in weight has not been checked.

Sept. 26, 1913: for the past four days there has been a general gradual rise in temperature to 102 F. The patient complains of very severe pain in her left lower axilla. The skin of the axillary region between the seventh and ninth ribs is exquisitely tender to touch. Physical examination and Roentgen-ray fail to reveal a cause for the pain and tenderness. Neither herpes nor subcutaneous emphysema is present. This patient had her first hemoptysis after the establishment of a pneumothorax. Complete compression checked further bleeding.

Case 10.—Schoolgirl, aged 18, single, American, admitted April 16, 1913 (Stage III). She had been a patient at Bedford during the fall and winter of 1911. After leaving the sanatorium she felt well until three months ago when her cough and expectoration returned. For the past three weeks she has had evening rises of temperature to 104 F. She presented marked involvement of her left lung with signs of a cavity beneath the left clavicle (Stage III). The right apex anteriorly was dull, the breathing was bronchovesicular and fine râles were heard after coughing (Stage I). Promptly after her first inflation on May 23, her temperature dropped to below 100 F. With complete compression her cough and expectoration were reduced and the patient felt well enough to leave her bed. With the establishment of a complete pneumothorax, the displacement of her heart to the right so terrified her that she refused further treatment.

Sept. 26, 1913: Ten weeks after treatment. Her compressed lung has reexpanded.

Case 11.—Woman, aged 39, married, Hungarian, housewife, admitted Aug. 5, 1912 (Stage III). For a year previous to admission she had suffered from cough and pain in the lower left chest. Eight months after admission pneumothorax was recommended for the excessive cough and expectoration, the attacks of pyrexia and progression of the disease on the left side (Stage II). There were only slight signs of involvement at the right apex posteriorly (Stage I). After three inflations a fairly well-marked compression of the left lung was evident. The cough and expectoration were diminished, the temperature practically normal and the patient felt better. May 28, 1913, when an inflation was attempted, the patient presented symptoms (profuse cold perspiration, pallor, nausea, a desire to urinate and defecate and a choking sensation in the throat) similar to those described as resulting from pleural shock or eclampsia. Her skin became cold and clammy. She was pulseless for about one minute. This occurred after the introduction of the needle, but before any gas had been allowed to enter. Following this attack she complained of

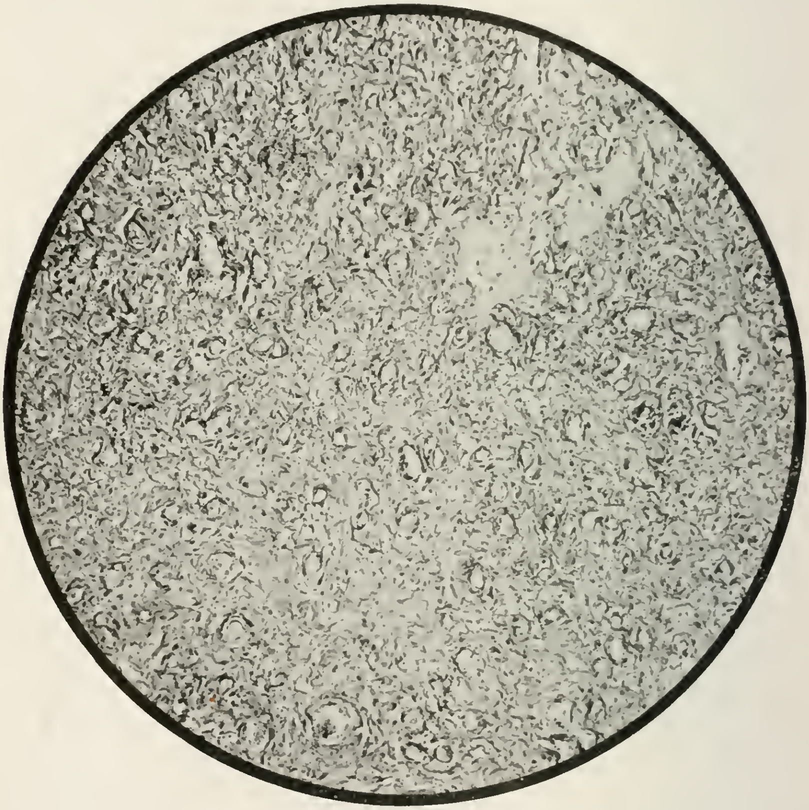

Fig. 9.—Conglomerate tubercles showing active organization, some completely replaced by young connective tissue. From a case of pulmonary tuberculosis (Case 4 of this series) in which artificial pneumothorax was induced.

pain in her lower left axilla (an effusion developed later) and has since failed to respond favorably to treatment. Her cough has again become troublesome. (She had tuberculous ulcers on right vocal cord and right arytenoid.) Fever and weakness have reappeared. There has been no evidence of increased activity on the untreated side. Owing to the development of a pleural effusion the treatment was discontinued.

Case 12.—Schoolgirl, aged 16, American, admitted Feb. 3, 1913 (Stage III). One year previous to admission she had suffered an attack of right-sided pleurisy. Recently she has had cough, expectoration and fever. On admission her general condition was very poor, her temperature ranging between 100 and 103 F. The entire right lung was consolidated (Stage III). Over the right lower lobe bronchophony and moist râles were heard. The left lung was not involved. The patient lost ground very rapidly and in the hope of relieving some of the symptoms, especially the pyrexia and cough, we decided to try the influence of pulmonary compression in spite of the pneumonic-like process in her right lung. We experienced considerable difficulty at the initial and all subsequent inflations, owing to her pleural adhesions. We finally succeeded in introducing 300 c.c. of nitrogen on May 14, 1913. The drop in temperature was prompt. Within two weeks the change in the clinical picture was striking. The patient remained afebrile. Her cough, expectoration, appetite and weight improved so that she was able to leave her bed.

June 11, 1913, 500 c.c. of nitrogen were introduced. Pain, which for a moment was sharp, was complained of during the inflation; this, however, subsided rapidly. Examination following the treatment revealed an area between the seventh and ninth spaces in the left axilla suggestive of a pneumothorax. This finding has been confirmed by both fluoroscopy and roentgenography, both showing a bilateral pneumothorax (Fig. 5). The case continued, however, to progress favorably for two or three weeks until the left lung began to give evidences of involvement, when improvement ceased, the temperature rose and the cough and expectoration returned. In spite of the appearance of the disease in the left lung and the bilateral pneumothorax, the patient is more comfortable, and the right lung shows less activity than before the institution of the treatment.

Case 13.—Woman, aged 20, single, Russian, cloakmaker, admitted Nov. 4, 1912 (Stage III). She gave a history of cough, expectoration and weakness for six months before admission. In spite of sanatorium care she constantly lost ground. In the spring of 1913 the left lung was extensively involved with a large cavity in the upper lobe (Stage III). The right lung showed slight infiltration in the upper lobe (Stage I). The loss of weight had been progressive and the pulmonary process was spreading actively. The temperature ranged between 99 and 102 F. An artificial pneumothorax was induced April 30, 1913. In three weeks' time a marked improvement occurred in both her subjective symptoms and general appearance, although the amount of compression obtained was relatively small. Improvement continued for another month and then the symptoms slowly returned. The involvement on the right side increased. Treatment, therefore, was suspended. The fluid obtained from a small complicating left-sided effusion was inoculated into a guinea-pig with a positive result.

Case 14.—Woman, aged 21, single, Austrian, neckwear worker, admitted Sept. 16, 1912 (Stage III). Three years' history of pulmonary tuberculosis. The patient had received two years' sanatorium care and a course of tuberculin treatment right lung (Stage I); left lung (Stage III). There was a cavity in the upper left lobe and fine crepitations were heard from apex to base. We made eleven unsuccessful attempts to introduce nitrogen into the pleural cavity and finally tried the Brauer method in vain. The interesting feature in connection with this patient occurred at several of our attempts to establish a pneumothorax. We frequently obtained well-marked oscillations in our manometer with negative pressure readings at the beginning of treatment. After about 100 c.c. of nitrogen had been introduced the manometer would suddenly show wide oscillations about zero point as a center. At no time were we able to discover gas in the pleura, although we frequently made a fluoroscopic examination immediately after treatment. We were unable to detect the escape of gas through her mouth. It is difficult to interpret the preceding manometric readings when they occur constantly. We were inclined to explain them by assuming that at each inflation the needle would enter a pleural pocket and that this would rupture after a small amount of gas had been introduced and the gas thereafter would flow directly into the lung.

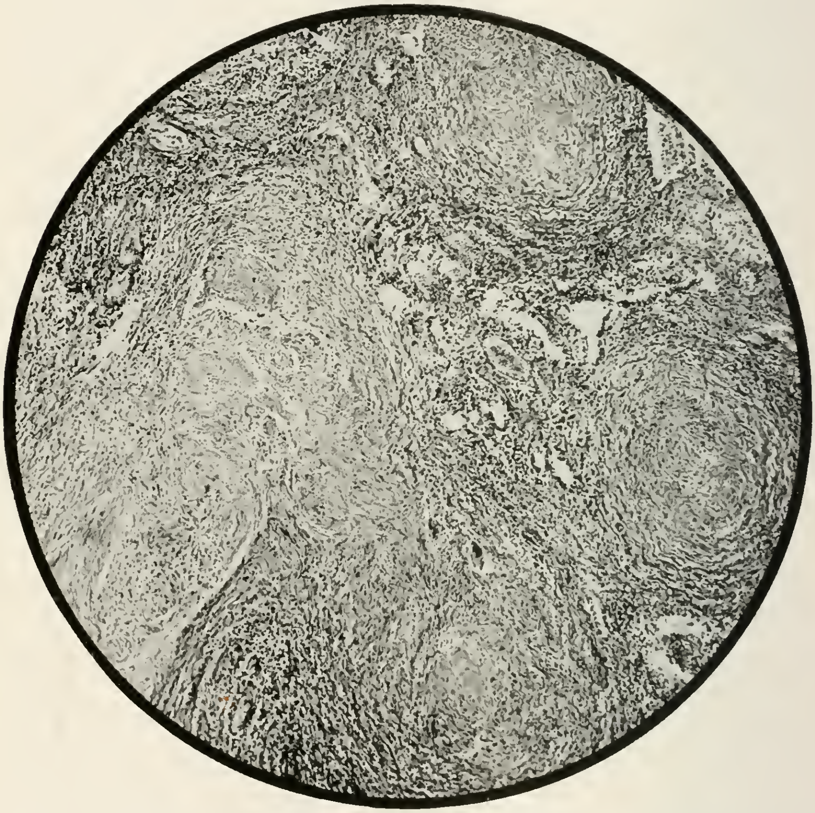

Fig. 10.—Large areas of the lung completely replaced by granulation tissue. From a case of pulmonary tuberculosis in which artificial pneumothorax was induced.

Case 15.—Woman, aged 20, single, Austrian, saleswoman, admitted November, 1911. (Stage III.) We simply wish to record this case as one in which a number of attempts to enter the pleural cavity were unsuccessful.

Case 16.—Woman, aged 40, married, Russian, seamstress, admitted April 9, 1912. (Stage III.) She stated that for two years prior to admission she had been troubled with a slowly increasing cough, expectoration, pain in chest and dyspnea. These symptoms have in the past three months become very troublesome. The patient presented a bilateral pulmonary process. The right lung (Stage III) showed moderate activity. The left lung (Stage I) was quiescent.

The pneumothorax was induced in the hope of controlling the excessive cough and expectoration. She remained under treatment for three and a half months, during which time good compression of the left lung was secured with marked relief from symptoms. She left the institution against advice.

Case 17.—Man, aged 34, married, American, admitted Nov. 28, 1912. (Stage II.) Before admission he had three large hemorrhages at various intervals. Since admission he has been afebrile. April 6, 1913, he had a large hemorrhage of about 40 ounces; two days later another of 15 ounces, and on the two following days 10 ounces and 8 ounces respectively. April 16 and 17 he expectorated blood again, in moderate quantities; likewise on May 25 and 26. His weight in April, 1913, was 156 pounds, a gain of 5 pounds since admission. There was extensive involvement of the entire right upper lobe, without excavation (Stage II) and slight percussion changes at the left apex (Stage I). On account of the previously recurring hemoptyses it was deemed advisable to compress his lung.

May 26 the first injection of gas, 500 c.c. were introduced without difficulty; manometric oscillations were negative and positive, of moderate degree, with zero as a center. There was no evidence of displacement of the heart, after this operation. On the following day the patient had a severe hemoptysis Auscultatory evidence of the presence of gas in the chest was lacking. May 28, 1,100 c.c. of nitrogen were introduced easily. June 3, 1,200 c.c. of nitrogen were introduced. June 12, 1,500 c.c. of nitrogen were introduced.

Previous to this time the patient was too ill to be moved to the Roentgen-ray room. After the last treatment, the fluoroscope showed that the base of the right lung was somewhat clear, but heart sound and râles were audible from apex down to base. Three days later a roentgenogram was made which showed no evidence of pneumothorax. June 17, 1,300 c.c. of nitrogen were introduced. The following day a fluoroscopic examination showed the right base somewhat clear. From this date to July 4 (about four weeks) the patient received five injections of gas in fairly large amounts and in spite of careful physical examination, no evidence of a definite pneumothorax could be established. Further treatment was therefore discontinued. It is probable that at each treatment the gas escaped into the lung.

Case 18.—(Fig. 7.) Man, aged 21, admitted May 29, 1913. (Stage III.) On admission physical examination of the chest showed bilateral involvement of the left side, and the roentgenogram showed infiltration in the upper half of the left and apex of the right lung. Temperature ranged between 98 and 103 F. The patient has had diarrhea since admission. Pulmonary compression was recommended on account of the poor prognosis, based upon the progression of the disease in the lungs, and the complicating diarrhea. July 7, the initial injection of 500 c.c. of nitrogen was introduced into the left pleural cavity. Following this there was a reduction in the temperature which soon became normal. During the following seven weeks, seven more injections were given varying in amounts from 500 to 1,300 c.c. and the treatment had to be discontinued because of severe pains in the lower left axilla and shoulder region (diaphragmatic pleurisy) and marked dyspnea. After the treatment had been discontinued, the temperature rose, varying from normal to 101.5 F. September 12, an effusion developed. The fluid was aspirated and a smear of the sediment showed no tubercle bacilli. Animal inoculation was not done in this case. The loss of weight has been progressive. There has been slight progression of disease on the right side. No improvement has occurred in the diarrhea.

Case 19.—Man, aged 21, single, American, clerk, admitted March, 1913. (Stage III.) There was continuous daily elevation of temperature up to 103 and 104 F., with severe chills and night-sweats. Weight on admission was 120 pounds. Patient was bed-ridden. There were present extensive involvement of the entire left lung (Stage III) and infiltration of the right apex (Stage I). (Fig. 8) with tuberculous laryngitis with aphonia (infiltration) as a complica-

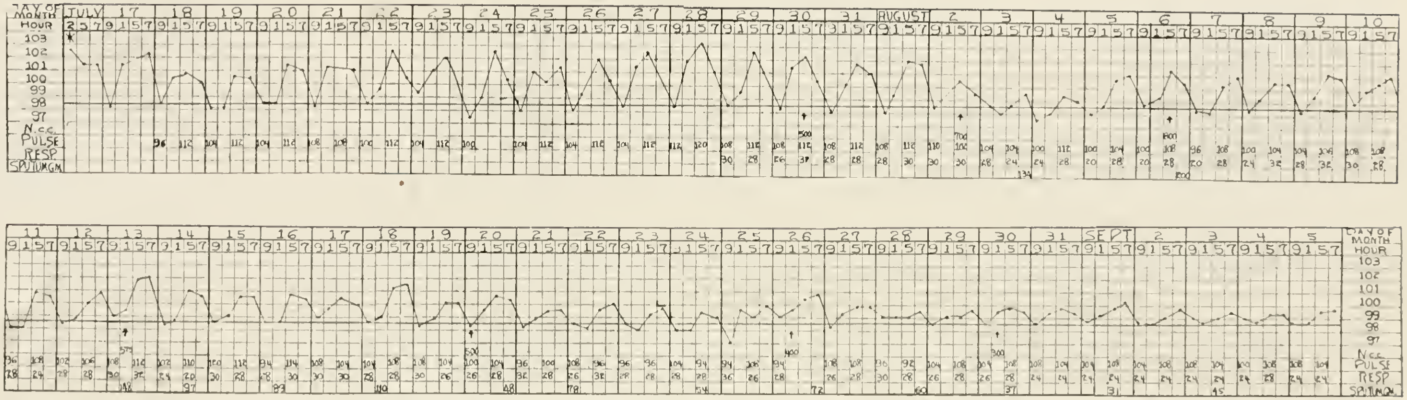

Fig. 11.—Chart of Case 3, showing reduction of temperature, pulse-rate and twenty-four-hour sputum output.

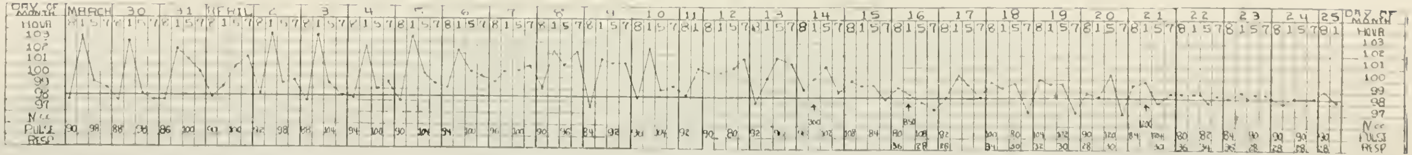

Fig. 12.—Chart of Case 19, showing prompt reduction of temperature following induction of artificial pneumothorax.

tion. The first injection of nitrogen, 300 c.c., was given April 14, 1913. The temperature began to drop promptly (Fig. 12). The chills and night-sweats ceased; the cough and expectoration lessened. The laryngitis improved and the patient's voice became clear. In the course of the first few weeks of treatment the patient's symptomatic improvement was marked. On the other hand, there was a slight progressive loss in weight.

May 1, patient was permitted to leave his bed and since then has been up and about and has made a trip to the city, a distance of forty miles. The pneumothorax was complete (Fig. 8).

Case 20.—Boy, aged 16, American, admitted October, 1912. (Stage III.) The temperature was continuously elevated, varying from 98 to 102 F., in the evening, usually around 100.5 F.; considerable cough and expectoration. He presented extensive disease of the left lung with excavation (Stage III). The right side showed only slight apical dulness (Stage I). The patient was recommended for pneumothorax treatment because of the progressive character of his disease.

June 30, first injection (800 c.c.) of nitrogen was introduced. The temperature has been normal since the beginning of treatment; there has been no cough or expectoration and no shortness of breath, in spite of the gas; the patient feels much stronger and has been exercising freely, playing ball, etc. There has been slight gain in weight. During August he received a total of 1,100 c.c. of gas on two separate occasions. September 18, he looks well, is cheerful and happy.

Case 21.—Man, aged 24, admitted July 15, 1912. Duration of disease thirteen months. (Stage III.) A severe hemoptysis of 45 ounces occurred April 15, 1913. For the following few days he had a slight evening temperature up to 100 and 101 F. On May 6 another hemorrhage of 10 ounces. Pneumothorax treatment was recommended because of hemoptyses. There was an extensive chronic process involving the entire left lung (Stage III) with slight percussion changes at the right apex (Stage I).

May 7, pneumothorax was instituted on the left side without difficulty, 800 c.c. of nitrogen being injected. May 12, patient feels well; expectoration diminished. May 21, fluoroscopic examination showed that the left lung was well compressed. June 4 and 11, fluoroscopic examination showed the left half of the chest to be very clear (gas) except for a marked shadow (lung) along the spinal column. Left half of the diaphragm was depressed. Heart displaced to the right. August 4, the left lung is well compressed. A slight pleural effusion has developed. A guinea-pig inoculated with the fluid aspirated, developed generalized tuberculosis.

Case 22.—Man, aged 26, single, schoolteacher, Russian, admitted March 18, 1913. (Stage III.) Considerable cough and expectoration were his most marked symptoms. There was extensive progressive involvement of the right side (Stage III).

Pneumothorax treatment was advised because of the extensive lesion and its progressive character. June 9, first injection, 350 c.c. nitrogen was introduced. Since the institution of pulmonary compression the cough and expectoration have been beneficially influenced. There has been some loss in weight. September 3, patient discharged at his own request. Condition improved.

Case 23 (Fig. 6).—Man, aged 24, Russian, admitted Nov. 4, 1912. (Stage III.) Duration of illness four years. The temperature has risen since admission, at times up to 104.5 F., but usually about 100 F. Considerable cough and expectoration have been present. The disease has been progressive. There was present extensive disease throughout entire left lung (Stage III) and right upper lobe (Stage II).

Pneumothorax treatment was advised because of the stage of the disease and its progressive character. May 5, first injection, 600 c.c. of nitrogen introduced. A pulmonary compression was maintained during the following four months. At the end of August the patient complained of pain in the left side and a moderate rise of temperature developed. A pleural effusion was suspected and was demonstrated by physical, as well as Roentgen-ray examination. Since the institution of the pneumothorax treatment the temperature had always been normal except for a period of one week, when the pleural effusion appeared and then the temperature ranged up to 102 F. A guinea-pig which was inoculated with the aspirated pleural fluid developed tuberculosis. The cough and expectoration were also diminished. The loss of weight was, however, progressive. There have been no night-sweats at any time during this period. September 25, physical signs in the right lung are unchanged. The left lung shows almost complete pneumothorax. There has never been any difficulty in getting into the pleural cavity. The patient has taken a trip to the city since treatment was instituted.

Case 24.—Man, aged 39, widower, American, duration of disease eight years, admitted 1912. (Stage III.) Since admission his temperature has ranged usually from 100 with rises to 102 and 103 F. about every ten days. The cough is severe and expectoration considerable, from 100 gm. to 150 gm. daily. He has lost weight rapidly, and his weakness has been marked. He presented extensive involvement of the left lung with a cavity in the lower lobe (Stage III) and infiltration of right upper lobe (Stage II). Because of the wide-spread disease and its progressive character, pneumothorax was recommended in the hope of staying the process somewhat, at least on the left side. April 28, the first attempt to produce a pneumothorax was unsuccessful. Good oscillations, negative and positive, were obtained, but always about zero as a center. Probably the gas escaped by way of the lung and bronchi. Fluoroscopic and physical examination failed to reveal any signs of gas. Four subsequent attempts to produce pneumothorax likewise proved futile. June 13, the first successful pneumothorax was established. The patient received 900 c.c. of nitrogen. July 15, 400 c.c. of nitrogen were given and subcutaneous emphysema developed. July 29, the patient developed a pleural effusion. The lung was only partially compressed. Apparently there was no diminution in size of the cavity on the left side. August 11, the left chest was aspirated. Fluid showed lymphocytosis of 95 per cent. A guinea-pig inoculated with the aspirated fluid was positive for tuberculosis. September 19, no change in the size of the pleural effusion. Up to date there has been no diminution in amount of the cough, expectoration or temperature. The loss of weight and weakness have been progressive. The pulmonary process has spread on both sides; a thick walled cavity on the left side prevented complete compression.

Case 25.—Man, aged 19, single, American, admitted April 17, 1913. (Stage III.) Duration of disease about one year. The patient has had temperature ranging constantly from 99 to 101.5 F. He showed extensive disease of the entire left lung anteriorly and of the left upper lobe posteriorly (Stage III). Pneumothorax was recommended on account of the unilateral extensive progressive disease. May 15, first injection of nitrogen (300 c.c.) was made; it was followed by a great deal of pain and dyspnea. The fluoroscopic examination showed a small clear area (pneumothorax) at the base of the left lung. Fifteen subsequent attempts to introduce nitrogen during the months of June, July and August were made and the largest amount of gas that could be introduced at any one time was 500 c.c., the usual amount being about 200 to 250 c.c. Further attempt to collapse the lung was discontinued owing to extensive pleuritic adhesions.

Case 26.—Man, aged 28, single, Austrian, admitted May 7, 1913 (Stage III); duration of disease several years.

The temperature since admission has ranged between 98 and 101 F., usually about 100 F. There was extensive bilateral disease, chiefly right-sided. Pneumothorax was recommended because of the widespread progressive disease.

June 16, the first injection was made (400 c.c. of nitrogen). Pulmonary compression was maintained during the following four months. September, temperature has been unaffected by the treatment, but the cough and expectoration have diminished slightly. There has been progressive loss of weight (15 pounds) in the past four months. The pneumothorax is complete. On the left side there has been a slight diminution in physical signs. The patient is sleeping better (due to the lessening of the cough), and his appetite is improved.

Case 27.—Man, aged 28, single, Russian, admitted Sept. 16, 1912. (Stage III.) Since admission the temperature has ranged up to 102 F. He has coughed and expectorated considerably. The disease has been progressive. Extensive disease of left lung (Stage III). Pneumothorax was recommended because of the wide-spread process in the left lung and its progressive character.

April 21, first injection was made (350 c.c. of nitrogen). During the following four months pulmonary compression was effectually maintained. There was no difficulty in entering the pleural cavity. After each inflation the patient complained of dyspnea and severe pains in the chest. Since treatment was instituted his temperature has been normal; he coughs and expectorates very little, and has made a trip to the city. His weight has been stationary.

Case 28.—Man, aged 27, single, Russian, admitted Jan. 1, 1913. (Stage III.) He has had repeated hemorrhages since admission and therapeutic pneumothorax was advised. His temperature has not been above 100 F. He presented extensive infiltration of the entire right lung (Stage III) and slight percussion changes at the left apex (Stage I).

June 13, first injection, 700 c.c. of nitrogen were introduced. July; during this month about 4,200 c.c. of nitrogen were injected. September, during the past five weeks patient received an additional 2,600 c.c. of nitrogen. He has had no hemoptysis since the institution of treatment. There has been no progression of the lesion on the left side. He has lost weight. His cough and expectoration remain uninfluenced.

Case 29.—Man, aged 21, Russian, single, admitted Feb. 24, 1913. (Stage III.) His temperature was normal, he coughed and expectorated moderately. There was extensive disease of the left lung (Stage III), with a dulness at the right apex (Stage I). Pneumothorax was recommended because of the extensive unilateral character of the disease. June 2, first injection, 600 c.c. of nitrogen, was introduced. August, he developed a left pleural effusion, which was aspirated. Examination showed a marked lymphocytosis of aspirated fluid; guinea-pig inoculated with the aspirated fluid showed tuberculosis. Pneumothorax in this case was successfully established. Improvement in the cough and expectoration followed. The patient's weight remained stationary. The signs in the right lung were unchanged.

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STUDIES ON ACIDOSIS AND DYSPNEA IN RENAL AND CARDIAC DISEASE *

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The present study was undertaken to obtain information concerning the cause of dyspnea in cardiac disease, with especial reference to the possibility of changes in the nature of the stimulus to respiration. Recent progress in our knowledge of the physiology of respiration and of its exciting stimulus, together with the development of certain physiological and chemical methods, have opened new pathways for the study of pathologic conditions.

The pioneer work of Haldane and Priestley seemed to establish the fact that the exciting stimulus to the respiratory center is the carbon dioxid content of the blood. This conception, however, has been broadened by such subsequent investigators as Winterstein[1] and Hasselbalch,[2] who have shown that the essential stimulus is the hydrogen-ion concentration of the blood. The variations in the carbon dioxid content of the blood, and consequently of the alveolar air, are but the result of the attempt on the part of the organism to keep the hydrogen-ion concentration of the blood at a constant value. The carbon dioxid in the blood rises as the amount of non-volatile acid falls, and the carbon dioxid falls as the production of other acids rises. L. J. Henderson[3] has been chiefly instrumental in showing the enormous biological importance of the constancy of the reaction of blood and other body-fluids, and in explaining the mechanism by which this constancy is maintained. In the living organism large amounts of the acid products of metabolism are continually being poured into the blood, but the constancy of the reaction of the blood is normally maintained, in part by the regulatory mechanism of certain remarkably adapted chemical equilibria (sodium carbonate—sodium bicarbonate, monosodium phosphate—disodium phosphate and the proteins) which allow the maximum amount of acid to be added with the minimum change in the reaction of the system, and in part by the process of elimination. The rôle played by the kidney has been especially studied by Henderson,[4] who has called attention to the remarkable specific

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[1] Winterstein: Arch. f. d. ges. Physiol., 1911, cxxxviii, 167.

[2] Hasselbalch: Biochem. Ztschr., 1912, xlvi, 403.

[3] Henderson, L. J.: Science, 1913, xxxvii, 389.

[4] Henderson: Jour. Biol. Chem., 1911, ix, 403.

action by which this organ is enabled to separate acid from its combined base, excrete the acid and save the base to the body so that it may again combine with and neutralize acid. The lungs form the second excretory channel of importance, for by means of them carbon dioxid is excreted. Any tendency to the production of an acidosis in the body is immediately compensated for by an increase in respiration and a washing out of carbon dioxid. It seems probable that the kidney is the fundamental regulator of the reaction of the blood, but the lungs, acting more quickly in response to sudden calls, serve as the more delicate balance-wheel of the regulatory mechanism.[5]

The question of the regulation of respiration has, however, been fundamentally widened by the writings of the two Danish physiologists, Hasselbalch and Lindhard, who have shown that there are two factors which must be considered. The first of these, and the one to which attention has hitherto been generally directed, is the nature or size of the stimulus to respiration. The second is the excitability of the respiratory center. This has been usually accepted as being a constant, and the possibility of its varying has been rather overlooked. To quote Hasselbalch's own words:[2]

> The two factors, regulation of neutrality and excitability of the respiratory center, together govern the chemical regulation of respiration, and therefore the amount of the alveolar carbon dioxid tension is a function of the two factors, and cannot be regarded as a physiologic constant."[6]

Hasselbalch states that a diminution in the carbon dioxid tension of the alveolar air is thus not necessarily due to an increase of the acids in the blood, since this condition may be present when the respiratory center is hyperexcitable and responds to a hydrogen-ion concentration of the blood that is lower than normal. In such a case the threshold for the excitation of the center has become lowered, but the composition of the blood as far as acids other than carbon dioxid go may remain unchanged. That variations in the excitability of the respiratory center do actually occur has been shown. The excitability may be tested by the method of Lindhard[7] in which the subject is given mixtures containing various percentages of carbon dioxid to breathe, and the increase in alveolar ventilation which results from a given increase in the inspired carbon dioxid is measured. Lindhard[7] has shown that the excitability of the respiratory center varies inversely as the oxygen tension of the blood. The excitability is furthermore increased by exposure of the person to ultra-violet light (Hasselbalch[2]), and in pregnancy (Hasselbalch[8]), but it is decreased by the action of morphin (Hasselbalch[2]). The decrease in the alveolar carbon dioxid tension described by several observers as occurring at high altitudes has been asserted by Hasselbalch and Lindhard[9] to be the result of an increase in the excitability of the respiratory center which is probably associated with the action of ultra-violet rays. Haldane and his coworkers[5] have criticized the experimental methods used by Lindhard in his work on the effect of oxygen tension on the excitability of the respiratory center. Their own experiments show no changes of excitability corresponding to variations in the oxygen tension, and they conclude that the excitability appears to be constant during health.

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[5] Campbell, Douglas, Haldane and Hobson: Jour. Physiol., 1913, xlvi, 301.

[6] Die zwei Faktoren: Neutralitätsregulation und Reizbarkeit des Atemzentrums beherrschen zusammen die chemische Atmungsregulation, so dass die Grösse der alveolaren CO_2 — Spannung als Funktion der beiden Faktoren hervorgeht und nicht als physiologische Konstante betrachtet werden kann.

[7] Lindhard: Jour. Physiol., 1911, xlii, 337.

Inasmuch, then, as blood, urine and alveolar air are all more or less directly related to the control of respiration, it is evident that in studying the complicated processes at work under pathologic conditions, all three of these factors should be considered.

In a consideration of the subject of cardiac dyspnea, one has various clinical types of patients under observation, and it is important to separate and simplify the types as far as possible. Perhaps the broadest lines that can be drawn are in the recognition on the one hand of the cases of uncomplicated cardiac disease, and on the other, cases of so-called "cardiorenal" disease in which the kidneys may be assumed to be the essential or at least an important underlying factor.

It is quite possible that different causes for the dyspnea in various types of uncomplicated heart-disease may be ascertained, and the study of large numbers of cases may lead to the recognition of definite groups of functional derangement. Of more immediate importance, however, is the determination of the rôle played by renal disturbance. It has long been known that certain types and stages of nephritis may be associated with the development of a considerable degree of acidosis, and since there is a direct relationship between acidity and respiratory control, it is logical to give some attention to this factor, and to determine if possible whether renal insufficiency has any bearing on the production of dyspnea. The first group of cases to be reported are, therefore, instances of renal disease in which cardiac involvement was either absent or of secondary importance. The second group of cases are instances of heart-disease with either no renal disturbance except passive congestion, or else a very moderate degree of nephritis.

It is to be regretted that the number of cases reported is not great, but some of them have been studied rather extensively, and as further investigation has necessarily been temporarily postponed, the work is reported in its present state.

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[8] Hasselbalch: Skandin. Arch. f. Physiol., 1912, xxvii, 1.

[9] Hasselbalch and Lindhard: Skandin. Arch. f. Physiol., 1911, xxv, 361.

METHODS

Alveolar Air.—The samples of alveolar air were almost always taken by the Plesch method. A comparative study of various methods of taking samples of alveolar air by Boothby and Peabody[10] showed the simplicity and reliability of this method for work with patients. Subsequent use has fully confirmed their opinion. Three separate samples were taken, and almost always a very satisfactory agreement within 2 mm. was found. Occasionally the carbon dioxid tension in one tube fell so far below the others that it was obviously an error, and this figure was discarded. The gas analyses were made with the small Haldane gas-analysis apparatus. The tension of the carbon dioxid in the alveolar air in normal persons usually ranged between 39 mm. and 44 mm.

Hydrogen-Ion Concentration of the Blood.—This was determined by means of the concentration cell, since titration and colorimetric methods do not give satisfactory results with blood. The general arrangement of apparatus was exactly like that described by Sörensen.[11] A platinum wire, calibrated in the physical laboratory of Harvard University, and a standard cell whose voltage was checked in the same laboratory, were used. An Edison nickel accumulator, and a Lippmann capillary electrometer were used. The hydrogen was generated in a Kipp apparatus and passed through a pyrogallic acid-potassium hydroxid solution, sulphuric acid, calcium chlorid tube and water before it entered the electrode vessel. All parts of the apparatus were mounted on paraffin blocks to prevent trouble from poor insulation. The electrodes were controlled by the use of the Sörensen standard phosphate solutions.

The measurement of the electromotive force, and thus of the hydrogen-ion concentration of simple solutions is comparatively easy by means of the concentration cell. The problem is, however, much more difficult when one is working with blood. This is on account of two features—the high albumin content of blood and the important part played by the carbon dioxid. The albumin content of blood apparently affects both the accuracy with which the readings can be made, and the rapidity with which an end-point is reached. The carbon dioxid is of even greater significance, for the reaction of blood varies according to the carbon dioxid tension at which it is tested.

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[10] Boothby, Walter M., and Peabody, Francis W.: A Comparison of Methods of Obtaining Alveolar Air, The Archives Int. Med., 1914, xiii, 497.

[11] Sörensen: Ergebn. d. Physiol., 1912, xii, 393.

Hasselbalch[12] has done an important service in calling attention to the error inherent in the previous work of most investigators of the reaction of blood, in that they neglected the escape of carbon dioxid, and he proposed a method by which it was possible to measure the hydrogen-ion concentration of blood saturated with carbon dioxid at a known tension. By the use of an ingeniously devised electrode vessel he is able to introduce, one after another, several samples of blood. Each new sample is shaken up in the atmosphere of hydrogen which is present in the electrode. Carbon dioxid escapes from the blood into the atmosphere above, but after several changes of blood, the atmosphere has a carbon dioxid tension equal to that of the blood, so that on the admission of a new sample, there is no loss of carbon dioxid. This form of electrode was used in the earlier work here, but it was found impossible to get constant results. After each change of blood there would be a fall in the reading and it was extremely difficult to get a constant end-point. Hasselbalch's[13] latest modification of his method, which is supposed to obviate some of the difficulties, was not tried, as it seemed much simpler to change the apparatus so as to be more on the principle of the method described by Michaelis and Rona.[14] The essential feature of this consists in the use of an electrode vessel which shall contain a large amount of blood and a small volume of hydrogen. With this arrangement only a comparatively small proportion of the carbon dioxid in the blood will pass out into the hydrogen atmosphere, and after a single filling of the vessel, a reading is obtained which is to all intents and purposes accurate. This does away with the changing of the blood necessary in the Hasselbalch vessel, in which the volume of blood is very small in proportion to the volume of hydrogen atmosphere. In the form finally adopted, the electrode vessels were shaped something like small Florence flasks with a tube for the inlet of blood set into the bottom, and a tube for the inlet of hydrogen set into the side of the neck. When ready for use, the bulb contains about 5 c.c. of blood and the neck about 1 c.c. of hydrogen. The neck is closed by a tightly fitting rubber stopper, through which is passed a piece of glass tubing with a short platinum wire, to serve as the electrode, fused into the end of it.

The blood used was taken from a vein at the elbow and defibrinated. Lundsgaard[15] has shown that the hydrogen-ion concentration is the same in defibrinated as in herudinized blood. The blood was then filtered and placed in a bottle through which was passed a rapid stream of a mixture of carbon dioxid and air. The mixture was analyzed for every experiment, so it was known at what tension of carbon dioxid the blood was saturated. After thorough saturation by running in this mixture for thirty minutes, and after thorough shaking, the blood was transferred by means of a glass syringe, and with constant care to prevent loss of carbon dioxid, into the electrode vessel. This vessel had previously been filled with the same mixture of carbon dioxid and air, so that there was no opportunity for the blood to lose carbon dioxid while it was being passed into the electrode vessel. The vessel and the side tube were completely filled with blood so that no bubbles of air remained. Then the side tube was connected with the hydrogen apparatus and blood allowed to run out of the lower end of the vessel until the neck of the vessel was filled with hydrogen, and the platinized electrode was just dipping into the blood. After shaking for ten minutes to promote a more rapid diffusion of gases, the electrode was allowed to stand. Readings were then made from time to time until a constant end-point was reached. Sometimes this occurred quickly, but usually it took two or three hours. All observations were made in duplicate, and at room temperature. The results were calculated and the temperature corrected to 18 C. (64.4 F.), using the formulas which are given in Sörensen's article.[11] The results are expressed in the now familiar terms of Sörensen as the ph.[16] It is to be remembered that the more alkaline a solution, the higher the ph., and the more acid, the lower the ph. When saturated at 40 mm. carbon dioxid the ph. of normal blood is approximately 7.45. Taking the high and low limits the extreme variations are between 7.40 and 7.50.

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[12] Hasselbalch: Biochem. Ztschr., 1910, xxx, 317; Hasselbalch and Lundsgaard: Biochem. Ztschr., 1912, xxxviii, 77.

[13] Hasselbalch: Biochem. Ztschr., 1913, xliv, 451.

[14] Michaelis and Rona: Biochem. Ztschr., 1909, xviii, 317.

[15] Lundsgaard: Biochem. Ztschr., 1912, xli, 247.

In all instances the ph. of the blood was determined when it had been saturated with a mixture containing carbon dioxid at about 41 mm. tension. This carbon dioxid tension may be considered as that at which the blood is saturated in the body when the alveolar carbon dioxid is normal. In normal individuals, therefore, it gives the actual ph. of the blood. If, however, the carbon dioxid in the alveolar air is below normal, as in cases of acidosis, the saturation at 41 mm. carbon dioxid will give a more acid value for the blood than is actually present. This is at least true in the majority of instances, for we know that the reduction of the alveolar carbon dioxid is usually an indication of the compensatory mechanism by which the blood-reaction is kept constant with a ph. of 7.45. The ph. of the blood saturated at 41 mm. carbon dioxid will thus be an index of abnormal acid formation. As such an index, it confirms what the determination of the alveolar carbon dioxid gives us, but it is also of especial value in cases in which it is not possible to obtain samples of alveolar air. Moreover, at present there has not been sufficient correlation of the alveolar carbon dioxid findings with the hydrogen-ion concentration of the blood in pathologic conditions for us to know in how far they correspond, as they do in normal conditions. In many instances, determinations of the ph. were also made on the same specimens of blood saturated with carbon dioxid at or about the tension at which it was found in the alveolar air. This would tell the actual reaction of the blood in the lungs. Usually this gives, of course, a ph. of the value of normal blood, about 7.45. Occasionally, however, in terminal conditions there is evidence of a failure of the compensatory mechanisms and of a true change in the reaction of the blood toward the acid side.

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[16] "Aus Zweckmässigkeitsgründen, die besonders bei graphischen Darstellungen der Abhängigkeit zwischen dem Verlauf biologischer Prozesse und der Wasserstoffionenkonzentration des Mediums hervortreten hat S. P. L. Sörensen vorgeschlagen als Mass für die Grösse der Wasserstoffionenkonzentration einfach der numerischen Wert des Exponenten der oben erwähnten Potenz von 10 zu benutzen und die Bezeichnung PH. anzuwenden. . . ." "Unter dem Wasserstoffionenexponenten, PH., einer Lösung ist der Brigg'sche Logarithmus des reziproken Wertes des auf Wasserstoffionen bezogenen Normalitätsfaktors, der Lösung zu verstehen." Sörensen: Ergebn. d. Physiol., 1912, xii, 401.

Urine.—The nitrogen was ascertained by Kjeldahl's method, and the ammonia determinations were made by the method of Folin.[17] The hydrogen-ion concentration of the urine was found by the colorimetric method described by Palmer.[18] The "retained base" and "total acidity" were determined by Adler and Blake's method.[19] To save space a great part of the urinary analyses are omitted, as they contribute little that is not shown by the other methods.

Blood.—I am indebted to Dr. C. Frothingham, Jr., and to Dr. W. G. Smillie, who kindly made the determinations of the non-protein nitrogen in blood by the method of Folin.[20]

CHRONIC NEPHRITIS

From the point of view of this study, the factor of importance in chronic nephritis is the development of acidosis. The cases examined fall clearly into two groups—those of uncomplicated nephritis and those of nephritis with uremic manifestations. Palmer[21] and others have shown, and studies in this hospital have confirmed the fact, that a very mild grade of acidosis is a frequent accompaniment of chronic nephritis. This is shown by the high hydrogen-ion concentration of the urine, by the total acid excretion, and by Palmer's sodium bicarbonate tolerance test. Apparently, however, in uncomplicated cases this degree of acidosis is taken care of by the kidneys. The increased excretion of acids in the urine serves to prevent any accumulation in the blood, and there is no decrease in the tension of the carbon dioxid in the alveolar air as there would be if the regulatory mechanism of the kidney were unable to compensate for the increased formation of non-volatile acids. Table 1 gives the alveolar carbon dioxid in a few cases of uncomplicated nephritis. It will be noted that the phthalein output in some of them indicates a nephritis of considerable severity.

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[17] Folin: Am. Jour. Physiol., 1905, xiii, 45.

[18] Palmer, Walter W., and Henderson, Lawrence J.: Studies Upon Acid Base Equilibrium and the Nature of Acidosis, The Archives Int. Med., 1913, xii, 153.

[19] Adler, Herman M., and Blake, Gerald: The Retention of Alkali by the Kidney with Special Reference to Acidosis, The Archives Int. Med., 1911, vii, 479.

[20] Folin and Denis: Jour. Biol. Chem., 1912, xi, 527.

[21] Palmer: Med. Communicat. Massachusetts Med. Soc., 1913, xxiv, 133.

TABLE 1.—Alveolar Carbon Dioxid in Chronic Nephritis without Cardiac Decompensation or Uremia

Case	Date	Non-Protein Nitrogen in Blood	Alveolar CO_2 mm.	Phthalein Output in Two Hours Per Cent.)
V. R......	11–13	....	44.1	
	12– 6	....	42.0	
J. J. B.....	12–19	....	42.7	
G. U.......	2–2	....	38.5	
J. J. F.....	2–3	47.6	40.2	25
	2–4	....	39.3	
	2–5	....	39.3	
	2–7	56.1	38.1	18
G..........	2–5	28.4	42.3	

The second group of renal cases consists of those in which there were definite manifestations of uremia. The majority of these ended fatally. In some of them it was possible to obtain observations over a more or less protracted period, during which the development of a marked acidosis could be followed. In these cases the determination of hydrogen-ion concentration of the blood was of great value, for after the patients became very sick, or developed coma, it was not possible to obtain samples of alveolar air.

Case 1.—H. G. M. (medical No. 621), woman, aged 28, was admitted to the hospital, Dec. 9, 1913, discharged Dec. 16, 1913. Diagnosis: chronic nephritis. There was no history of any previous illness, but the patient gave an indefinite account of rheumatic fever a few years ago. Onset of present illness is somewhat obscure, but apparently she had had disturbance of vision for over a year. During the summer she was well enough to play tennis several times, but had to give it up owing to "flashes" before her eyes. Two months before admission she had a severe attack of nausea and vomiting, and she has had some nausea since. There has been no edema other than a slight swelling of the eyelids.

Physical examination was generally negative, except for a hypertrophied heart. Blood-pressure: systolic 280 +. The eye-grounds show several hemorrhages and marked albuminuric retinitis.

Urine: acid, specific gravity 1.012, albumin, 0.3 per cent.; sediment, few hyaline, many granular and blood-casts.

Blood: hemoglobin 81 per cent.

The phthalein test was made twice during her stay in the hospital, and on both occasions only traces appeared in the urine.

December 10: Uncoagulable nitrogen in the blood, 90 mm. per 100 c.c. The ph. of the urine varied from 5.7 to 7.

During her stay in the hospital the patient vomited frequently and had several severe headaches. It was quite apparent that these were early symptoms of uremia. After leaving the hospital she developed marked muscular twitching and slowly lost ground. She died about ten days after leaving the hospital.

The ph. of the blood determined at 41.8 mm. tension of carbon dioxid December 11 was 7.44. This day the carbon dioxid tension of the alveolar air was 40.3 mm. Carbon dioxid tension of alveolar air December 12, 41.7 mm. and December 13, 40.4 mm.

This case then, at the beginning of what proved to be a terminal uremia, showed a perfectly normal carbon dioxid tension of the alveolar air and correspondingly a normal hydrogen-ion concentration of the blood.

Case 2.—L. F. C. (medical No. 655), man, aged 30, admitted Dec. 19, 1913, died Jan. 10, 1914. Diagnosis: chronic interstitial nephritis. This patient has been admitted to the hospital three times for chronic nephritis. The only etiological factors in the past history are malaria and dysentery, which he had in the Philippines ten years ago. For nine years he has had headaches, but these have been more severe in the last year and a half. On each occasion he has entered the hospital with a history of morning headaches, polyuria, moderate edema, poor vision, and slight dyspnea. Only on the final admission was there any evidence of grave cardiac insufficiency. At the last admission he was having marked dyspnea and orthopnea. The urine always showed a large amount of albumin and a low specific gravity. On the first admission the phthalein output was 20 per cent. in three hours, on the second admission 8 per cent. in two hours, and on the last admission it was zero in two hours. Blood-pressure has ranged from 200 to 240. Since the first admission there has been an albuminuric retinitis. There was cardiac hypertrophy but there were no murmurs except for a loud systolic murmur best heard in the aortic area, which developed under observation a few days before death. The dyspnea and orthopnea which were evidences of cardiac insufficiency and were present on his final admission, disappeared shortly after he was put to bed. After the first few days the heart was apparently completely compensated. Thus, December 21, the note states that "the dyspnea which was marked on entrance has disappeared. He breathes easily and sleeps lying almost flat. There is marked pallor of the skin and mucous membranes. Respiratory rate is 21. There is slight irregularity in rhythm and depth. Lungs are negative except for scattered râles, especially at the bases. Heart-sounds are loud and clear; no murmurs heard."

December 24, the patient began to be restless and his mind became clouded. From that time he gradually failed with increase of mental clouding. He vomited frequently and was at times definitely delirous.

January 9: "The patient seems very dopey at this time but responds when told to put out his tongue. There is marked pallor. There is no dyspnea and no orthopnea. Respirations are irregular but there are no definite periods of apnea. Lungs are clear on percussion, but the breath-sounds are distant. Heart is enlarged. There is no edema. Pulse-rate 88. There is constant muscular twitching."

January 10: Patient sank into unconsciousness and died. Diagnosis of chronic nephritis confirmed by necropsy.

TABLE 2.—FINDINGS IN CASE 2. CHRONIC NEPHRITIS ENDING IN UREMIA

Date,	Volume Urine c.c.	Ph. in Urine	Non-Protein Nitrogen in Blood mg. per 100 c.c.	H-ion Concentration of Blood		Tension of Alveolar CO_2 in mm.	Remarks
				Tension of CO_2 in mm.	Ph.		
1913							
12/19	...	5.5	....	42.7	7.37	36.3	Marked dyspnea. Pallor. Phthalein = 28 per cent. in two hours.
12/20	2,100	5.25	96.1	.....	....	35.8	
12/21	2,420	4.9					
12/22	3,110	4.85	....	.....	....	39.0	Blood-pressure = 230 mm.
12/23	2,300	5.00	....	.....	....	37.7	
12/24	1,930	5.05	....	.....	....	35.6	
12/25	.....	5.05	....	.....	....		
12/26	1,065	5.1	....	42.0	7.45	35.4	
12/31	1,150	4.9	....	.....	....	37.7	
1914							
1/ 1	1,430	4.7	....	.....	....	38.9	
1/ 2	1,590	4.8	129.8				
1/ 3	500	4.8					
1/ 4	1,160	4.9					
1/ 5	920	....	....	.....	....	39.1	Phthalein output = 0.
1/ 6	0	....	....	.....	....	41.3	Blood-pressure = 230 mm.
1/ 7	750	4.7	278.9	40.7	7.45	38.8	
1/ 8	560	4.7*	330.0	45.4†	7.15	....	No dyspnea.
			....	41.0‡	7.21	36.8	Blood-pressure fallen to 180 mm.
1/ 9	.....	....	347.0	41.0	7.04	....	Too sick to get alveolar air.
1/10	.....	....	....	.....	....	....	Died at 4 a. m.

*Below; † a. m.; ‡ p. m.

The studies of the urine during the first few days show little that is significant. During the last days it was impossible to collect twenty-four-hour specimens, so that the figures are not of great value. The hydrogen-ion concentration, however, as one would have expected, tended at all times toward low limits. The non-protein nitrogen of the blood shows a progressive increase, reaching at the end the extremely high value of 347 mg. per 100 c.c. blood. The determinations of the hydrogen-ion concentration of the blood and of the carbon dioxid tension of the alveolar air are of considerable interest. On admission when there was marked dyspnea the alveolar air was slightly below normal, and the ph. of the blood also indicated a very slight degree of acidosis. Following this the carbon dioxid in the alveolar air rises, but the figures are rather irregular and slightly low. December 26, the ph. of the blood was normal. January 7, both the blood and the alveolar air were normal. January 8, however, at about the time at which severe symptoms of uremia developed, there was a slight fall in the carbon dioxid tension of the alveolar air and a marked fall in the ph. of the blood. A comparison of the alveolar air and the blood-findings suggests strongly that at this time the actual reaction of the blood had become abnormally acid. January 9, the patient was so sick that it was impossible to get the alveolar air, but the ph. of the blood showed a very marked acidosis. Perhaps the most striking result of these studies is the fact that at the period when dyspnea and orthopnea were marked, both blood and alveolar air showed very little evidence of acidosis, while at a period when acidosis was extreme there was scarcely any disturbance of the respiration.

Case 3.—N. H. (medical No. 617), woman, aged 56, admitted Dec. 8, 1913, died Dec. 12, 1913. Diagnosis: chronic nephritis, uremia. The patient was unconscious when she was brought into the hospital. At a subsequent date a history of kidney trouble was obtained. The present condition apparently came on acutely about sixteen hours before admission to the hospital.

Physical examination showed an obese woman, lying in deep coma. Respiration rather difficult and loud. No cyanosis. Heart apparently not markedly enlarged. Pulse 72. Blood-pressure, systolic 120, diastolic 75. Lungs show fine and coarse râles. Abdomen, negative. No edema. Eye-grounds, negative.

Urine, specific gravity 1.021, albumin, trace; sediment, great numbers of hyaline and a few granular casts.

Non-protein nitrogen of the blood: 65.7 mm. per 100 c.c.

December 8: Phthalein test. No urine obtained at the end of two hours by catheterization. Hydrogen-ion concentration of the blood at 40 mm., carbon dioxid tension = 7.39.

December 10: Patient can be roused to answer questions. There is almost complete anuria. Two catheterized specimens of urine were obtained of 35 c.c. and 18 c.c., respectively, during the twenty-four hours.

December 11. Patient is in deeper coma. Blood taken ten hours before death, non-protein nitrogen, 133 mg. per 100 c.c. of blood. The ph. of the blood saturated at 38.7 mm., carbon dioxid tension = 7.29.

December 12: At 8 p. m. last evening the patient lapsed into deeper coma with stertorous respiration. This morning she had general convulsions and died. Necropsy showed chronic nephritis.

Urine examinations could not be made on this patient as the output was so small. It was impossible to obtain specimens of alveolar air. The hydrogen-ion concentration of the blood, however, was determined on two occasions. When the patient was admitted the hydrogen-ion concentration was practically normal, but December 11 there was evidence of well-marked acidosis. Shortly after this determination was made the patient developed a deep, slow respiration, somewhat suggestive of the respiration seen in diabetic acidosis.

Case 4.—J. W. D. (medical No. 673), man, aged 32, admitted Dec. 26, 1913, died Jan. 4, 1914. Diagnosis: chronic nephritis, uremia, pericarditis. Complaint, vomiting. Past history negative, except for osteomyelitis of the tibia twelve years ago. For six months he has had frontal headache in the morning. This has been often associated with vomiting. During the last three weeks he has been short of breath on exertion and has had to prop himself up in bed at night. He has also been very drowsy; has had epistaxis for two months. No edema. Vision is normal. He worked as usual until four days ago.

Physical examination shows a fairly well-developed young man lying propped up in bed breathing rapidly with some effort. Skin, pale; heart: left border of dulness in the fifth space, 11 cm. from the midsternal line. There is a high-pitched systolic murmur at the apex. Aortic second sound is rather weak. Blood-pressure: systolic 180, diastolic 125. There is moderate effusion in the pleural cavities. Reflexes very active; no edema.

Urine acid, specific gravity 1.026, trace of albumin. Sediment negative.

December 30: Output of phenolsulphonephthalein is zero for two hours. This has been repeated twice. Non-protein nitrogen of blood is 209 mg.

January 1: Pericardial rub noticed. Patient is perfectly clear mentally. Output of urine 800 c.c.

January 2: Mind is clear, no headache. Nausea and vomiting continue.

January 3: Respiration 12 per minute, regular, slightly deep but not abnormally so. There is muscular twitching. Mind perfectly clear. Urine output 350 c.c. Carbon dioxid tension of alveolar air, 33.8 mm. The ph. of the blood measured at 39.8 mm., carbon dioxid tension, 7.32. Non-protein nitrogen of the blood 285.7 mg. per 100 c.c.

January 4: Patient became unconscious during the night and died early in the morning. Necropsy showed a chronic nephritis, acute pericarditis and edema of the lungs.

In this case it was impossible to make complete urinary examinations. The alveolar air and the hydrogen-ion concentration of the blood determined January 3, correspond extremely well and indicate a moderate degree of acidosis. At this time the respiration was slow and regular and there was no evidence of dyspnea.

Case 5.—L. C. M. (medical No. 573), woman, aged 38, admitted Nov. 29, 1913, died Dec. 7, 1913. Diagnosis: chronic nephritis, uremia. With the exception of an attack of measles as a child, the patient has never had any illness confining her to bed. For the past five years she has had headaches. These began as mild attacks once a month, and have gradually increased in frequency and severity, now occurring weekly. She has been dyspneic on exertion for the past five years, but has never had to leave work. One week before admission she had a severe "bilious" attack, with pain in the epigastrium and vomiting. She has had continued nausea since the onset of this attack, but no other disturbance until three days before admission. At this time she noticed that she could not see objects clearly. On admission she could not count fingers at a distance of four feet. She has noticed that she has passed very little urine during the last week.

Physical examination shows a small, undersized, poorly nourished woman. There is no marked dyspnea. Eyes and pupils are normal; the pupils are equal and react normally. There is a conjunctival hemorrhage on the temporal side of the right eye. Mucous membranes are pale. Breath is foul. Heart: apex indefinitely localized in the sixth space, 7 cm. from the midsternal line; left border is 12 cm. to the left of the midsternal line in the sixth space, and the right border is 3.5 cm. to the right of the midsternal line. There is a loud, blowing systolic murmur at the apex. Aortic second sound is markedly accentuated and there is a long systolic murmur heard over the aortic area. Pulse-rate 90. Blood-pressure, systolic 230, diastolic 130. Lungs show a few râles at the right base; slight dulness with râles at the left apex. Examination of the eye-grounds shows well-marked albuminuric retinitis.

Urine acid, specific gravity 1.012; albumin, large trace; sediment contains many hyaline and a few granular casts, rare red blood-corpuscle.

Blood: Hemoglobin, 66 per cent.; leukocytes, 14,600.

November 30: During the night the patient was bled about 450 c.c. Although her mental condition was perfectly clear, it was feared that uremia was impending owing to the condition of the eye-grounds and the urinary findings, together with the absence of any output of phenolsulphonephthalein. The patient has been put on a milk diet of 1,500 c.c.

TABLE 3.—Findings in Case 5. Chronic Nephritis Terminating in Uremia

Date, 1913	Volume Urine c.c.	Ph. in Urine	Non-Protein Nitrogen in Blood mg. per 100 c.c.	H-ion Concentration of Blood		Tension of Alveolar CO_2 in mm.	Remarks
				Tension of CO_2 in mm.	Ph.		
11/29	...	....	90.5	.....	....	....	Headache, vomiting. Albuminuric retinitis. Phthalein = 3 per cent. in two hours.
11/30	210	7.4 +	....	.....	....	....	Phthalein = 0 in two hours.
12/ 1	250	7.4 +	....	.....	....	32.9	
12/ 2	260	Menses	....	.....	....	31.4	Phthalein = 0 in two hours.
12/ 3	150	Menses	117.0	.....	....	29.1	Eyesight almost gone.
12/ 4	170	Menses	200.0	.....	.....	26.1	
12/ 5	200	7.4	....	42.5	7.22	....	Mind clear.
			....	34.4	7.27		
12/ 6	58 *	7.4	....	43.7	7.10	....	Blood taken six hours before death. Mind cloudy at 6 p. m. Unconscious at 11 p. m.
			....	21.8	7.23	24.4	
12/ 7	...	....				....	Died at 2:50 a. m.

* Catheter specimen.

December 1: The patient complains only of general weakness. Headache is less and there is no edema.

December 2: Patient mentally clear and does not complain of headache.

December 3: Vision is, if anything, decreased. There is practically no dyspnea and no edema. During the past twenty-four hours the urine output has not risen above 200 c.c. in spite of two doses of theocin.

December 4: The patient was bled about 400 c.c. last night. Following this, 600 c.c. of normal salt solution was introduced per rectum. Warm bath and later a hot pack given. Patient seemed somewhat better. There are no subjective symptoms whatever at this time other than the blindness, which is now almost complete.

December 7: The patient's condition up to 6 p. m., December 6, was unchanged. At that time her mental condition was somewhat clouded. In spite of venesection and enteroclysis, her condition became worse during the early part of the evening and at 11 p. m. she became unconscious. Death occurred at 2:50 a. m.

The preceding case is thus one of pure chronic nephritis terminating in uremia. During the whole of her stay in the hospital the excretion of urine was very small. The non-protein nitrogen in the blood was markedly increased and rose progressively. In the last two tests there was no output of phenolsulphonephthalein in two hours. The urine examination shows a high ammonia percentage, which would suggest acidosis. It is interesting, however, that the hydrogen-ion concentration of the urine was at all times low. There was nothing in the diet or medication to account for this unusually low acidity. Analyses of the alveolar air showed a progessively falling carbon dioxid content. The ph. of the blood was determined on the last two days and on both occasions shows evidence of marked acidosis. Indeed the determination December 6, of blood taken six hours before death, shows evidence of a true shift in the reaction of the blood toward the acid side. It is to be noted that at no time during the course of the disease was dyspnea noticed.

DISCUSSION OF FOREGOING CASES

The foregoing cases confirm the work of von Jaksch,[22] who, from results obtained by a rather crude titration method, was the first to describe the acidosis of uremia. More recent investigators, using more accurate methods, have added little that is of essential importance. Straub and Schlayer,[23] studying the carbon dioxid tension in samples of the alveolar air, obtained by the Haldane method, found a decreased tension in eight cases of uremia. Slightly low values were found in a number of cases showing only moderate uremic symptoms. The lowest tension occurred in the most advanced cases. These authors go so far as to assume a causal relationship between the acidosis and the uremic condition. Cases of nephritis without evidence of uremia showed normal carbon dioxid tension. Similar results were obtained by Porges and Leimdorfer,[24] who also made determinations of the carbon dioxid of the alveolar air. Poulton and Ryffel[25] found the alveolar carbon dioxid tension between 14 and 25 mm. in four cases of uremia. The urea-content of the blood was high, and the lactic acid in the blood slightly increased. The blood of these patients took up oxygen with abnormal difficulty in spite of the low pressure of carbon dioxid present. "This effect would be produced by the presence of excess of acid in the blood." These authors add that "as there was the possibility that the meionexy * in these cases might be directly connected with the high percentage of urea in the blood, a control experiment was made in which 0.39 per cent. urea was added to normal blood." The results showed that the addition of urea to blood does not cause the dissociation curve to shift to the right.

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[22] Von Jaksch: Ztschr. f. klin. Med., 1888, xiii, 350.

[23] Straub and Schlayer: München. med. Wchnschr., 1912, lix, 569.

[24] Porges and Leimdorfer: Ztschr. f. klin. Med., 1913, lxxvii, 464.

[25] Poulton and Ryffel: Proc. Physiol. Soc., June 28, 1913, published in the Jour. Physiol., 1913, xlvii, p. xlvii.

There are also a number of direct determinations of the hydrogen-ion concentration of the blood in nephritis with and without uremia. Thus Kreiblich[26] found that in uncomplicated nephritis the reaction of the blood was within normal limits, while in uremia there was an acidosis. Rolly[27] also found that the hydrogen-ion concentration of the blood was normal in nephritis without uremia. In four cases of terminal uremia there was evidence of acidosis, but in three cases with temporary uremia the blood was normal. He believes that an acidosis of the degree found in uremia may be present in the agonal stages of many conditions. The findings of these last two observers are open to the criticism that the determinations of the hydrogen-ion concentration of the blood were not made in blood which had previously been saturated at a known carbon dioxid tension. We have already seen that this is essential in order to obtain accurate values.

The present studies confirm in general the work of previous investigators, but they also give more accurate information as to the relation of the acidosis to some of the associated conditions. The evidence is certainly against the theory adopted by Straub and Schlayer that acidosis is a cause of uremia. Several of the cases show that marked symptoms of uremia may exist without any evidence of acidosis as manifested by the alveolar air or by the blood. That slightly low figures for the carbon dioxid tension in the alveolar air may be obtained in some mild cases of uremia, as found by Straub and Schlayer, and by Porges and Leimdorfer, is certain; but all the evidence goes to show that acidosis of any great importance is rarely found except in severe and usually terminal cases. Even in cases in which the blood was examined within twelve hours before death, the acidosis is not so marked as is found in advanced diabetic coma. In one case of diabetic coma in this hospital the blood saturated at 43.2

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*Lewis, Ryffel, Wolf, Cotton and Bancroft: The condition in which the blood takes up less than its usual share of oxygen is called *meionexy;* the opposite condition, that in which it takes up more than its usual share, is called *pleonexy-meionexy,* indicates a shifting of the reaction in the acid direction," Heart, 1913, v, 45.

[26] Kreiblich: Wien. klin. Wchnschr., 1911, xxiv, 1419.

[27] Rolly: München. med. Wchnschr., 1912, lix, 1201.

mm. carbon dioxid had a ph. of 7.07. The development of acidosis in renal disease bears little or no relation to the amount of non-protein nitrogen in the blood. The latter may be extremely high without signs of marked acidosis being present. This is confirmatory of the experiments of Poulton and Ryffel.[25] Furthermore, the relation is slight between the output of phenolsulphonephthalein and the development of acidosis. In several instances the phthalein output had fallen to zero some time before evidence of acidosis arose. It is thus only after the excretory power of the kidney has been reduced to a minimum for a somewhat prolonged time that the signs of acidosis begin to develop in the blood. Finally there is apparently little relation between the occurrence of acidosis in nephritis and that of dyspnea. Dyspnea on exertion was observed in some of these cases a long time before there was any development of acidosis, and on the other hand, at the time when acidosis was present, respiratory disturbance was certainly not a prominent feature. Occasionally a type of deep, slow respiration is seen, resembling the respiration of typical diabetic acidosis. It bears, however, little relation to the type of breathing in cardiac dyspnea. It seems thus fair to conclude that in combined cardiorenal disease, the dyspnea is essentially a circulatory feature, and the renal condition plays an accessory part.

CARDIAC DYSPNEA

There are many researches concerning the nature of cardiac dyspnea, but comparatively few deal specifically with the nature of the stimulus to respiration. Beddard and Pembery[28] found the carbon dioxid in the alveolar air reduced in decompensated cardias cases. They are uncertain whether the stimulus to rapid respiration is chemical or nervous in origin. Lack of oxygen appeared not to be the cause, as the administration of oxygen had little effect. Fitzgerald[29] also studied the alveolar air in cardiac disease and found values that are rather low in most of the causes in which dyspnea is noted.

A much more extensive study was made by Porges, Leimdörfer and Markovici.[30] These authors found that the carbon dioxid in the alveolar air in cases of cardiac disease without dyspnea is normal. In cases with dyspnea they found that the tension of alveolar carbon dioxid is usually diminished. In a few instances, a normal tension was found and this they explain by the presence of an associated pulmonary condition, for their observations go to show that in dyspnea due to pulmonary disease the tension of alveolar carbon dioxid is increased. The average tension in thirty-six cases of cardiac disease without dyspnea was 43.5 mm. if the barometric pressure is assumed to be 76.5 mm. The results are given only in percentages. The average of thirty-one observations on cases with dyspnea was 33.0 mm. Porges assumes that the low carbon dioxid in the alveolar air is the result of an abnormal formation of acid bodies and concludes that the dyspnea in cardiac disease is due to an acidosis.

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[28] Beddard and Pembrey: Brit. Med. Jour., 1908, ii, 580.

[29] Fitzgerald: Jour. Path. and Bacteriol., 1910, xiv, 328.

[30] Porges, Leimdörfer and Markovici: Ztschr. f. klin. Med., 1913, lxxvii, 446.

A much more complete study of dyspnea in cardiac and renal patients has lately been published by Lewis, Ryffel, Wolf, Cotton and Barcroft.[31] They call attention to the fact that while patients with congenital heart-lesion may have marked cyanosis without dyspnea, the majority of patients with definite breathlessness are not particularly cyanotic. The degree of cyanosis certainly does not correspond with the degree of dyspnea, so they conclude that the dyspnea is not wholly accounted for by lack of oxygen. In the cases without marked cyanosis they believe that the cause of the dyspnea is a relative acidity of the blood—an acidity which is not due to excess of carbon dioxid. In the cases with deeper cyanosis, the acidosis is an etiological factor acting in addition to the lack of oxygen. The group of cases in which dyspnea occurred with only slight or moderate cyanosis were for the most part those in elderly persons with enlarged hearts, with or without valvular disease, often with arteriosclerosis or renal involvement. This group they term the "special cases" in contradistinction to a group of three "cardiac cases." In only one of the latter does the record state that there was "deep cyanosis." In the other two it was said to be "very slight" and "slight or moderate"— descriptions also found applied to some of the "special cases." Studies of the oxygen-combining power of the blood showed an acidosis (meionexy) which is more often definitely present in the "special cases." The determinations of the carbon dioxid in the alveolar air fall into two groups. In the nine "special cases" the values are below normal. Of the two "cardiac cases" one had a normal and one a rather increased carbon dioxid. They feel that the meionexy in the "cardiac cases" may be accounted for by carbon dioxid, but that in the "special cases" it must be due to an increase of non-volatile acids. With a special test for the determination of non-volatile acids, they found that all the "special cases" gave evidence of an increase, while the two "cardiac cases" examined did not. It is to be noted, however, that five of the eleven "special cases" showed as little or less increase in non-volatile acids than did two of the three "special control cases" which had "no dyspnea but little reserve." The table also shows that this test for non-volatile acids does not always run parallel to the depression of carbon dioxid tension in the alveolar air. Thus, for example, Patient 1 had a lactic acid equivalent of 0.15 per cent. and an alveolar carbon dioxid of 26.0 mm. Patient 3, with a lactic acid equivalent of 0.15 per cent., had an alveolar carbon dioxid of 13.0 mm., and Patient 5, with a lactic acid equivalent of 0.007 per cent. had alveolar carbon dioxid tension of 28.0 mm. Improvement of general conditions with lessening of dyspnea in the same case was associated with a diminution of the acidosis. Determinations of urea, non-protein nitrogen and chlorids in the blood showed that these substances had little relation to acidosis. An increase of lactic acid in the blood was present in only two terminal cases, and there was no evidence of increase of lactic acid in the urine. No connection was found between the acidosis and urinary excretion of ammonia or total acid. Since the urine examinations were negative these authors believe that the "acidosis appears to be another instance of the same phenomenon of retention of acids in excess of bases which is shown during residence at high altitudes." "During residence at an altitude of 10,000 feet or more the alveolar carbon dioxid falls progressively until accommodation is established. An excess of non-volatile acid is retained in the blood to compensate for this loss of carbonic acid, with the result that the dissociation curve of the blood is slightly displaced in the acid direction. The slight increase of lactic acid which is observed in the blood is not adequate to account for more than a small part of this retention of acid and no increase of lactic acid is excreted in the urine, so that this change appears to be an instance of the second cause of acidosis, namely, altered excretion of acids and bases by the kidney, which is beneficial at high altitudes, as the pressure of oxygen in the lungs is thereby increased."

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[31] Lewis, Ryffel, Wolf, Cotton and Barcroft: Heart, 1913, v, 45.

CASE 6.—J. W. (medical No. 828), man, aged 37, admitted Feb. 7, 1914. Diagnosis: chronic valvular disease, mitral insufficiency, mitral stenosis, aortic insufficiency, auricular fibrillation. This patient had previously been in the hospital for the same condition. Five days after having been discharged from the hospital, the patient was admitted again on account of intense dyspnea, precordial pain, and pain in the right hypochondrium.

There is marked cyanosis of the lips and lobes of the ears. Lungs: negative except for a few moist râles at both bases. Heart: left border 17 cm. to the left of the midsternal line in the sixth space; right border is 5.5 cm. to the right. At the apex there is a blowing systolic murmur following the first sound and a faint diastolic rumble. Along the left border of the sternum there is a distant blowing diastolic murmur. Pulse is absolutely irregular. Blood-pressure: systolic 130, diastolic 85. Liver dulness extends from the sixth rib to 8 cm. below the costal margin. There is tenderness over the liver. No ascites, no edema of the extremities.

February 9: Patient is still slightly dyspneic and there is a little cyanosis of lips, ears and fingers. Examination of the heart is essentially as on admission. Urine: specific gravity, 1.021; albumin, trace; sediment, rare hyaline cast.

February 11: Dyspnea has almost entirely disappeared. Patient complains of no subjective symptoms Urine: specific gravity 1.014; albumin, 0; sediment negative.

February 16: Under digitalis the pulse-rate fell to 40 per minute. Since then it has increased in rate to 60. Dyspnea has entirely disappeared.

February 20: Patient was suddenly seized with intense sharp pain, which was referred to both lower legs. Subsequent examination showed evidence of an embolus of the posterior tibial artery.

Case 7.—J. T. (medical No. 787), man, aged 16, admitted Jan. 26, 1914, discharged Feb. 9, 1914. Diagnosis: chronic valvular disease, mitral insufficiency, mitral stenosis, auricular fibrillation. This patient has been in the hospital twice before. He was readmitted on account of marked dyspnea.

Physical examination shows considerable cyanosis. Lungs: negative except for a few râles in the left axilla, and at each base posteriorly. Heart: left border 14 cm. to left of the midsternal line in the fifth space, right border 5 cm. to the right in the third space. At the apex both sounds are replaced by murmurs. There is a loud, blowing, systolic murmur transmitted to the left axilla and a rumbling diastolic murmur. Toward the sternum in the sixth space there is a louder, higher-pitched systolic murmur. The pulmonic second is greatly increased. Pulse is absolutely irregular and 96 at the apex. Liver: lower border is indefinitely made out 8 cm. below the costal margin in the right nipple line. There is great tenderness on pressure over the liver. There is no ascites. Slight edema over both shins. Urine: acid; specific gravity 1.025; albumin, large trace; sediment, rare hyaline cast.

January 30: Cyanosis has almost disappeared. Respirations are 14 per minute. Patient has no dyspnea and feels perfectly comfortable.

Case 8.—H. N. (medical No. 719), woman, aged 46, admitted Jan. 10, 1914. discharged Feb. 7, 1914. Diagnosis: chronic valvular disease, mitral stenosis, mitral insufficiency. Patient has had two attacks of rheumatic fever, which were followed by heart-trouble. During the last two years she has had three breaks in compensation, which compelled her to go to bed. She has been short of breath for two years. She is not able to walk a block without getting "purple"; has been in bed for three weeks. Orthopnea at night.

Physical examination: lips, ears and mucous membranes are very cyanotic. Heart: left border 16 cm. to the left of the midsternum in the fifth interspace and 4 cm. to the right in the fourth interspace. There is a presystolic thrill at the apex. The first sound is followed by a systolic murmur which is transmitted to the axilla. Second sound is scarcely audible and is followed by a loud, rough, diastolic murmur ending with a presystolic accentuation. Over the tricuspid area the systolic murmur is much higher pitched and of different character than that heard at the apex. Pulmonic second sound is accentuated. Pulse is regular. Lungs: numerous crackling râles on inspiration and expiration. Abdomen: definite shifting dulness. Liver dulness extends from the sixth rib to 5 cm. below the costal margin. There is edema of the legs. Blood-pressure, systolic 128, diastolic 80.

January 11: Patient has marked cyanosis of face and hands. Respiration is not rapid. There is no dyspnea while she sits propped up. Examination of the heart is as at admission.

January 29: Patient is sitting up in a chair. There is very little shortness of breath and no edema. Lungs are practically clear.

January 10: Urine, specific gravity, 1.025; albumin, trace; sediment no casts, many white blood-cells. Blood hemoglobin: 95 per cent.; red cells, 4,570,000.

January 21: Urine, specific gravity, 1.020; albumin, very large trace; sediment, rare hyaline cast, many white blood-cells.

Case 9.—A. M. (medical No. 865), woman, aged 46, admitted Feb. 9, 1914. Diagnosis: chronic valvular disease, mitral stenosis, mitral insufficiency, auricular fibrillation. The patient had rheumatic fever at 14 years of age. For twelve years she has had heart-trouble, including many periods of broken compensation. She was discharged from this hospital Jan. 3, 1914, after a stay of seven weeks. Her condition is now about what it was at the previous admission. For the last week she has been extremely short of breath, with orthopnea and a sense of smothering at night. Abdomen and legs have swollen rapidly.

Physical examination shows very little cyanosis, but marked dyspnea. Respiration about 40 per minute. Heart: left border of dulness is 15 cm. to the left of the midsternal line in the fifth space; right border is 4 cm. to right. Action is absolutely irregular. The first sound at the apex is followed by a loud, harsh systolic murmur, and the second is followed by a loud, blowing, diastolic murmur. At the base both sounds are weak. Lungs: dulness to percussion at both bases with some diminution in the intensity of the breath-sounds; a few scattered râles. Liver: lower border 3 cm. below costal margin. Liver pulsating and very tender. Slight edema of legs.

Blood: hemoglobin, 97 per cent.; leukocytes, 6,600.

February 20: Urine, specific gravity, 1.020; albumin, large trace; sediment, many casts.

February 21: Patient feels very much more comfortable. Respiration easier. Color is good. Respiration is still rapid.

February 23: Looks much better. Respiration remains rapid. Blood-pressure, systolic, 135; diastolic, 80.

February 24: Urine, specific gravity, 1.018; albumin, slightest possible trace; sediment, rare hyaline cast. Phthalein output in two hours, 40 per cent.

Case 10.—I. J., man, aged 50, admitted Feb. 15, 1914. Diagnosis: acute rheumatic fever, chronic, valvular disease, mitral insufficiency, aortic stenosis (?). This patient gives a history of three previous attacks of acute articular rheumatism. For two years he has been troubled with dyspnea on exertion. He has had frequent attacks of what he terms "asthma" at night. When admitted to the hospital he had considerable dyspnea.

There was no cyanosis. Heart: left border in the sixth interspace is 18 cm. to the left of the midsternal line, right border is 3.5 cm. to the right. Action is rapid and absolutely irregular. The first sound at the apex is followed by a systolic murmur. The pulmonic second sound is accentuated. In the aortic area there is a rough systolic murmur, followed by a loud, snapping second sound. There is a coarse systolic thrill over the aortic area. Lungs: a few râles posteriorly. No emphysema. Liver not enlarged. There is an acute inflammation of the joints of the first finger on the right hand.

February 15: Urine, specific gravity, 1.025; albumin, slightest possible trace; sediment, few hyaline casts. Blood: hemoglobin, 80 per cent.; leukocytes, 13,000.

February 17: Dyspnea is very much less to-day; no cyanosis.

February 19: Urine, specific gravity, 1.026; albumin, 0; sediment negative.

Case 11.—M. R. (medical No. 837), man, aged 53, admitted Feb. 11, 1914, discharged Feb. 21, 1914. Diagnosis: chronic valvular disease, mitral stenosis. The past history has no bearing on the present condition. For three months the patient has noticed dyspnea on exertion. This has gradually increased in severity until at present any slight exertion practically exhausts him. He has had no edema.

On examination there is slight cyanosis of the lips and marked dyspnea, although the patient is able to lie flat on his back. Heart: left border 11.5 cm. to the left of the midsternal line in the fifth space; right border is at the right sternal margin. The action is regular. The first sound at the apex is loud and snapping, no definite systolic murmur heard. Second sound is distant and followed by a long, rumbling diastolic murmur with presystolic accentuation. Both sounds are weak at the base. Lungs: numerous fine, moist râles at both bases posteriorly. Liver not enlarged or tender. No edema.

Urine, specific gravity, 1.010; albumin, trace; sediment, occasional hyaline cast.

Blood, hemoglobin, 105 per cent.; leukocytes, 15,000.

The dyspnea disappeared gradually in a few days, and on discharge the patient's respiration was normal except for dyspnea on exertion.

Case 12.—A. J., man, aged 52, admitted Feb. 17, 1914. Diagnosis: chronic myocarditis. There is nothing in the past history of the patient which has any relation to the present disease. He was first admitted to this hospital Oct. 31, 1913, at which time he had very severe dyspnea. The dyspnea had persisted for about six weeks. There was no edema. November 11, the phthalein output in two hours was 55 per cent. He was discharged much improved November 12. Since his discharge he has become progressively more short of breath, and when admitted the second time was dyspneic and had Cheyne-Stokes respiration.

Heart: Left border 18.5 cm. to left of midsternal line in sixth interspace, right border 3.5 cm. to right in fourth interspace; action regular; rate 90. At the apex the sounds are fairly well audible, and there is a systolic murmur. Sounds are distant at the base. Lungs: moderate dulness with diminution in intensity of breath-sounds and a few râles at both bases posteriorly. Liver: edge felt 7 cm. below the costal margin. It is not tender. Much edema of legs. Blood-pressure: systolic, 130; diastolic, 98.

February 18: Urine, specific gravity, 1.026; albumin, trace; sediment, rare cells and granular casts. Blood: hemoglobin, 108 per cent.; leukocytes, 10.200. Phthalein output in two hours, 27.0 per cent.

February 23: The patient has had no cyanosis. He is rather pale. He has orthopnea. Cheyne-Stokes respiration has persisted and is rather more marked.

DISCUSSION OF CASES 6 TO 12

The number of cases reported here is not large, but the findings are all of the same general type and of the same order of magnitude as are reported in other analogous studies, so that they represent fairly well-recognized types of cases. It is comparatively easy to classify them according to the laboratory findings and by such a procedure they fall into two main groups. One of these is somewhat analogous to the "special" group of Lewis, especially in that the patients show a decreased alveolar carbon dioxid. In this series, however, the attempt has been made to rule out or take account of any renal element in the cases. The second group corresponds more or less to Lewis' "cardiac cases." Group 1 consists of Cases 6, 7 and 12, in which on the whole the highest grade of dyspnea occurred. Cases 6 and 7 are certainly as pure cardiac cases as one sees. Patient 12, with a slightly decreased phthalein output, probably has some renal involvement and arteriosclerosis. This group is characterized by a low tension of the alveolar carbon dioxid during the dyspnea period, which is followed by a rise when compensation is regained. The determinations of the ph. in the blood, however, show that the actual hydrogen-ion concentration of the blood is at or below the low limits of normal. Certainly the reaction of the blood does not tend to shift toward the acid side, although the considerable drop of the alveolar carbon dioxid in the cases makes it probable that there is some increase of non-volatile acids in the blood. The urinary ammonia was neither relatively nor absolutely increased. In one case there was a mod-

TABLE 4.—Nephritis with Cardiac Decompensation

Case	Date, 1914	Non-Protein Nitrogen mg. per 100 c.c.	H-ion Concentration of Blood		Alveolar CO_2 mm.	Phthalein Output in 2 Hours. Normal from 40 to 60 Per Cent.	Remarks
			Tension of CO_2 in mm.	Ph.			
J. W....	2/7	28.9	41.9	7.38	30.9	61.0	Marked cyanosis. Dyspnea. Blood-pressure, systolic 130, diastolic 85.
	2/8	....	....	....	30.8	....	Dyspnea and cyanosis less, but distinct.
	2/9	23.1	42.8 33.4	7.34 7.44	.... 33.6	....	Slight cyanosis. Much less dyspnea.
	2/10	....	....	....	36.4	....	Much more comfortable.
	2/11	18.1	44.6 37.7	7.44 7.51	.... 36.0	....	Respiration 17.
	2/12	....	....	....	39.4	....	Practically no cyanosis or dyspnea.
	2/15	....	....	....	40.9		
	2/17	22.1	....	....	41.1		
J. F......	1/26	34.0	42.0 35.0	7.50 7.53	.... 34.7	50.0	Considerable cyanosis. Moderate dyspnea.
	1/30	....	43.5	7.47	41.9	....	Slight cyanosis. Respiration 14. No dyspnea.
H. N....	1/13	....	....	....	37.8	40.0	Marked cyanosis. Respiration quiet 26. Dyspnea on exertion.
	1/14	....	....	....	38.3		
	1/15	....	....	....	37.7		
	1/16	....	....	....	39.3		
	1/19	....	....	....	37.4		
	1/20	31.4	39.7	7.45	38.4		
A. M....	2/19	20.7	43.5	7.45	4.44	....	Very marked dyspnea. Slight cyanosis. Respiration 30, labored.
	2/20	....	....	....	41.5	....	Very marked dyspnea. Very slight cyanosis.
	2/21	....	....	....	38.2	....	Respiration easier. Color is normal. Still has considerable dyspnea.
	2/23	....	42.3	7.50	41.2	40.0	Respiration still rapid, but not labored. No cyanosis. Blood-pressure = sys. 135, dias. 80.

TABLE 4.—Nephritis with Cardiac Decompensation — (Continued)

Case	Date, 1914	Non-Protein Nitrogen mg. per 100 c.c.	H-ion Concentration of Blood		Alveolar CO_2 mm.	Phthalein Output in 2 Hours. Normal from 40 to 60 Per Cent.	Remarks
			Tension of CO_2 in mm.	Ph.			
I. J......	2/16	35.7	42.0	7.40	46.0	48.0	Dyspnea. Respiration 35. Lies flat on back. No cyanosis. No especial pallor.
	2/17	....	....	....	42.1	....	Dyspnea less. No cyanosis. Has received 6.0 gm. $NaHCO_3$ since yesterday.
M. R.....	2/11	31.0	46.4	7.40	46.5	49.0	Moderate dyspnea, much increased by moving. Lies flat on his back. Slight cyanosis.
	2/12	....	....	....	46.7	....	Very little dyspnea or cyanosis. Blood - pressure, sys. 110, dias. 70.
A. J......	2/18	....	44.6	7.44	35.0	27.0	Very marked dyspnea and orthopnea. Very slight cyanosis. Moderate Cheyne-Stokes respiration.
	2/19	....	....	....	29.9	....	Cheyne - Stokes respiration.
	2/20	44.2	43.0	7.42	31.2	....	Cheyne - Stokes respiration.
	2/23	....	....	....	31.8	....	Cheyne - Stokes respiration. Has been given $NaHCO_3$.

erately high hydrogen-ion concentration of the urine (ph. = 4.6) and a slight increase of "retained base" which fell as compensation was established. The urine, however, showed no evidence of marked acidosis.

The second group consists of Cases 9, 10 and 11. These are characterized by a normal or very slightly increased carbon dioxid tension of the alveolar air. In two of the patients there was a tendency of the carbon dioxid to fall as compensation occurred. There was not enough involvement of the lungs to account for this rise in carbon dioxid on the basis of an interference with respiratory exchange. Cyanosis was not marked in any of them. The hydrogen-ion concentration of the blood saturated at the tension of alveolar carbon dioxid was normal. There was no evidence in either the blood or alveolar-air determinations of an increase in non-volatile acids in the blood. The rise in alveolar carbon dioxid which may possibly be regarded as abnormal was compensated, so that reaction of the blood was normal. In other conditions a much higher alveolar carbon dioxid may be found without causing dyspnea. Thus in a case of emphysema the alveolar carbon dioxid tension was 52.6 mm., the ph. of the blood saturated at 44.0 mm. was 7.56 and saturated at 53.4 mm. was 7.45. The patient showed no dyspnea.

Case 8 was one of mitral stenosis with considerable cyanosis, but little dyspnea, except when the patient moved. Both the alveolar air and the hydrogen-ion concentration of the blood were normal. Thus in the group of cases in which the severest dyspnea occurred, although the alveolar carbon dioxid is low, the hydrogen-ion concentration of the blood tends to be lower than normal. In spite of the fact that there is evidence of some increase of non-volatile acids, the respiratory center is responding to a stimulus that is apparently less than normal. The increased formation of non-volatile acids seems to be overcompensated by the decrease in carbon dioxid. In the second group of cases which showed more moderate dyspnea, the alveolar carbon dioxid is somewhat increased, but the hydrogen-ion concentration of the blood is normal. There was thus no evidence of an increase of non-volatile acids in the blood.

It is quite striking that in neither group of cases is there any evidence of a very high grade of acidosis. The same degree of depression of alveolar carbon dioxid is frequently seen in other conditions—uremia, diabetes—without producing any particularly obvious effect on the respiratory center. Thus in one case of diabetes, in which there was an ammonia output of 5 gm. in twenty-four hours, the alveolar carbon dioxid tension was 32 mm., while in a second case, in which there was a similar ammonia output, the carbon dioxid tension was only 26 mm. Neither of the patients had any disturbance of respiration which was evident on a routine clinical examination.

The degree of cyanosis makes it improbable that lack of oxygen is an important factor in the direct stimulus to this type of dyspnea. Since, then, there is apparently no very great change in the nature of the stimulus, one must seek some other condition which may play a part in causing this profound change in respiration. It is of course possible that the underlying factor is some as yet unknown substance which affects the stimulus. We have, however, already seen that in considering the mechanism of the control of respiration, it is necessary to take into account not only the stimulus to respiration, but also the excitability of the respiratory center. Porges[30] discards this latter factor, as he says that even Hasselbalch calculates that hyperexcitability of the respiratory center would not practically produce a fall in the alveolar carbon dioxid tension of over 5 mm. In the second group of cases, however, there is no fall in the alveolar carbon dioxid, and no especial change in the ph. of the blood so that here the theory of a hyperexcitability is quite tenable. In the first group of cases there is certainly evidence of some acidosis, not such a degree as would greatly affect the respiration normally, but one which, in the presence of a hyperexcitable respiratory center, might produce much more marked change in breathing.

There is nothing inherently untenable in the conception of a variable excitability of the respiratory center. Variations in the excitability of all nervous mechanisms are continually seen in both physiological and pathological conditions. Indeed, it is almost impossible to imagine the excitability of the controlling center of any physiological mechanism as being a constant. The experimental studies showing variations in the excitability of the respiratory center have already been referred to. It is certainly much more than probable that similar and perhaps greater variations may exsist under pathological conditions. Moreover, certain simple clinical facts point to the existence of such variations in decompensated heart-disease. Thus the dyspnea produced by moderate exertion can hardly be the result of a change in the stimulus alone. Similarly morphin, one of the most definite effects of which is to depress the excitability of the respiratory center, is certainly the most efficient drug in the control of cardiac dyspnea. A general consideration of the clinical facts and laboratory findings makes it highly probable that, while in some cases of cardiac dyspnea the stimulus to respiration is increased by the production of a moderate grade of acidosis, another important causative element is an increase in the excitability of the respiratory center.

It is hardly possible at present to offer an adequate explanation of the cause of an increase in excitability of the respiratory center. If, as Lindhard[7] considers to be proved, a diminution of oxygen tension increases the excitability of the center, one might consider that with a slowing of the circulation due to cardiac insufficiency the oxygen tension in the respiratory center becomes decreased, and the center becomes hyperexcitable so that it responds to a lesser stimulus than normally and more actively to a given stimulus. In very severe cases of cardiac insufficiency with a greater slowing of blood-flow there would be, added to this factor, a change in the stimulus itself due to the passage into the blood of acid substances—the partially oxidized products of metabolism. In a similar way, renal disease would be a contributing factor in the causation of dyspnea, for as soon as the kidney begins to fail in its function of acid excretion, the regulatory mechanism controlling the constancy of blood-reaction is upset, and there will be a tendency to call on the respiration to counteract the threatened acidosis. Haldane and his associates[5] have lately laid much stress on the fundamental function of the kidney in replacing the hydrogen-ion concentration of the blood and thus of indirectly influencing respiration. In so-called "cardiorenal" disease, the weakening of the myocardium would produce less adequate, slower circulation. One result would probably be an increased excitability of the respiratory center due to diminished oxygen tension in it. A second result would be an interference with renal excretion (as shown by the decreased output of phthalein during the period of decompensation) and the tendency to retain acids in the blood. The resulting acidosis while not necessarily of a grade to affect respiration in a normal person, may produce profound dyspnea in a patient with a weak heart. In cases of pure cardiac disease with decompensation and slowing of the circulation the lowered oxygen tension would increase the excitability of the respiratory center, and in more severe failure of the heart with the production of passive congestion there will be a temporary upset of renal function in the same way, although perhaps in a less degree than when kidneys are themselves organically diseased. Thus in simple cardiac disease, failure and slowing of the circulation may cause a disturbance in renal excretion, as well as an incomplete oxidation of abnormal acids. Both of these tend to produce an acidosis. The resulting change in the stimulus to respiration, even if it is slight, when acting on a respiratory center rendered hyperexcitable by an inadequate blood-flow, may well cause dyspnea.

In a somewhat similar manner Boothby[32] has recently suggested a slowing of the blood-flow as an explanation of the unusual condition of hyperpnea after forced respiration. He believes that the slowing may bring about a condition of oxygen-want, and in consequence, the presence of lactic acid or some similar exciting substance, which, in conjunction with the carbon dioxid, keeps the respiratory center excited so that the normal apnea does not appear. The only essential difference between this theory and the one put forward above is as to whether the oxygen-want resulting from an inadequate circulation shall be regarded as acting directly on the respiratory center by increasing its excitability, or as causing the presence of partially oxidized substances in the blood which are themselves added to the stimulus to respiration. It is to be remembered that in the cases of dyspnea studied by Lewis,[26] only a very slight increase in the lactic acid content was found, in other than moribund patients.

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[32] Boothby: Jour. Physiol., 1912, xlv, 328.

The direct determination of the excitability of the respiratory center under pathological conditions is planned as the next step in this investigation. It is quite possible that the excitability of the respiratory center may be an index of the functional adequacy of the circulation.

CONCLUSIONS

The mild grade of acidosis which is often associated with uncomplicated chronic nephritis is compensated for by the increased excretion of acids by the kidney.

It is only in uremia and usually only in very advanced uremia that non-volatile acids accumulate in the blood in an amount sufficient to cause depression of the tension of carbon dioxid in the alveolar air.

In the terminal stage of uremia there may be a high grade of acidosis.

The development of acidosis bears little relation to the accumulation of non-protein nitrogen in the blood or to the output of phenolsulphonephthalein. Determinations of both of these may show extreme abnormalities before any marked degree of acidosis develops.

The development of acidosis is not the direct or sole cause of dyspnea associated with cardiorenal disease. A high grade of acidosis may exist without causing a change in respiration which is evident on the ordinary clinical examination.

In some cases of cardiac disease accompanied by dyspnea there is evidence of a slight accumulation of non-volatile acids in the blood. In other cases there is no evidence of such an accumulation.

The tension of the carbon dioxid in the alveolar air is normal or very slightly increased.

The hydrogen-ion concentration of the blood remains normal.

In neither group of cases is the degree of acidosis sufficient to account for the dyspnea on the basis of an increase in the stimulus to respiration alone.

It seems probable that an important factor in the causation of dyspnea in cardiac disease is an increase in the excitability of the respiratory center. Such a change in excitability may well be caused by a lowering of the oxygen tension, and be dependent on an inadequate blood-flow.

I wish to express my gratitude to Miss B. I. Barker for her unfailing assistance in carrying out much of the analytical work on which this paper is based.

CLINICAL AND METABOLISM STUDIES IN A CASE OF MYOTONIA CONGENITA—THOMSEN'S DISEASE *

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I. INTRODUCTION

In this paper we present the clinical history of a case of myotonia congenita together with a metabolic study of the patient. The clinical side of the disease has been well summarized and the literature reviewed by Hale White.[1] Therefore we shall comment only on those features of the condition brought out by our metabolism study.

II. CLINICAL HISTORY OF CASE

Patient.—G. M., Austrian, male, single, aged 22, was admitted to the medical service of St. Francis Hospital, Aug. 12, 1912, complaining of stiffness and cramps in the muscles on first attempting movements.

Present Illness.—The present condition was first noticed by the patient at the age of 12 while a student at the gymnasium. At that time he became conscious of a stiffness about the knees and legs, and found that he could not take so active a part in the games as formerly. He experienced difficulty in walking, especially in going up and down stairs. This condition persisted with varying degrees of intensity until the age of puberty, when it became more severe and more generalized. During that period he found difficulty in speaking, in opening his jaws, in flexing his hand after making a fist, in protruding his tongue, and in extending his legs after flexion. Accompanying this stiffness there was a feeling of undue fatigue following any exertion, although generally at the beginning of any exercise he felt as strong as his fellows. A short rest would restore him to normal strength. The cramps were most pronounced when any movement was attempted while he was at rest. The repetition of any movement rendered it less difficult, and after a few attempts the stiffness would disappear and the movement become easy to perform except for the muscle fatigue which followed. The patient thinks that within recent years he has partially outgrown his trouble, and states that the condition is now less generalized, being at present largely confined to the hamstring muscles of the thigh and to the muscles of the arm, forearm and fingers. The ensuing fatigue is also less marked. He has still at times some uncertainty in walking, which he describes as a feeling that his knees are going to give way. There is always difficulty in starting to walk. He also complains of a pain which has the nature of a dull ache passing up and down his legs, most noticeable behind the knees. There is more or less general pain, which he refers to the regions of the larger joints of the body. When at rest all of his pain disappears. At times he becomes very irritable and nervous, especially at night, when he sometimes

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* From the Wards of St. Francis Hospital and the Biochemical Laboratory of the University of Pittsburgh.

* A preliminary account of this study was published in Proc. Soc. Exper. Biol. and Med., 1913, x, 136. By mistake the title reads "myotonia atrophica," instead of "myotonia congenita."

[1] White, Hale: Guy's Hosp. Rep., London, 1889, xlvi, 329 (contains good bibliography); Allbutt and Rolleston's System of Medicine, London, 1910, vii.

shakes all over. He has been under the care of physicians since the onset of his trouble, but has never obtained any benefit from treatment.

Family History.—The patient knows little concerning his ancestors, and was unable to give any satisfactory account of a similar condition existing in his family save in the paternal grandfather, who was troubled with similar cramps in his hands, having the same difficulty in opening up his hand after flexing his fingers. The father died during the patient's infancy. One aunt was nervous and had a tremor.

Past History.—The patient was born in Austria, and lived there until coming to America two years ago. As a child he had the usual diseases of childhood; otherwise he was always fairly healthy. He has been subject to frequent attacks of headache for years, and occasionally has ringing and buzzing in the ears. For about three years he has been obliged to wear glasses; he says that he is unable to read small print or to read in the presence of bright sunlight. He takes cold readily and has had frequent attacks of tonsillitis. At times he has shortness of breath and palpitation following exertion and is occasionally conscious of a dull pain over the precordium, which he describes as a feeling of tightness as though a vise were pressing on his heart. He denies venereal infection, and has noticed a hard mass on his right testicle for the past two years. He works as a baker, and takes about three glasses of wine a day and an occasional beer. He smokes moderately, has a fair appetite, eats regularly and sleeps poorly.

Present Condition.—The patient is a rather pale, but fairly well-nourished young man, with intelligent expression; he weighs about 147 pounds. The skin is moist and of normal color. The mucous membranes are pale. The pupils are equal, regular, somewhat dilated, and react well to light and accommodation. The sclerae are clear and palpebral slits equal. There is no hemianopsia. On extreme outward rotation of the eyeball there is a slight but distinct lateral nystagmus. The eyegrounds are negative on ophthalmoscopic examination. The nasolabial folds are equal. The tongue is protruded in the midline. The thyroid is not enlarged. A few posterior cervical glands are just palpable. The thorax is well formed; expansion good and equal. Lungs are negative. Cardiac dulness is normal. The heart sounds at apex are somewhat muffled; no murmurs. Shortly after admission to hospital a few extrasystoles were noted, but these later disappeared, and the rhythm became regular. The pulse is 72 to the minute, good in force and volume. The vessel wall is not thickened. The abdomen is natural and walls everywhere soft; abdominal reflex active, spleen and kidneys not palpable and hepatic dulness normal. There is a hard mass connected with the right testicle; genitalia otherwise normal. Over anterior tibial surfaces are some pigmented areas. There are no periosteal nodes. Some hyperesthesia of soles is present. There is no glandular enlargement. Epitrochlears are not palpable.

The knee-jerks are almost completely abolished. (This reflex, although frequently tested, was obtained only twice during the patient's stay in the hospital.) The biceps and triceps reflexes cannot be elicited; Babinski negative.

The special senses of hearing, taste and smell are normal. Tactile sensation is good over entire body. Stereognosis and joint sense are normal. Patient writes well. There is no scanning speech or other speech defect, except for an occasional slurring of the r's. The gait is negative but there is always difficulty in starting. Romberg negative.

Muscular System.—There is everywhere good muscular development, and in certain parts of the body some muscle groups, as the biceps brachii and pectorals suggest an appearance of hypertrophy. The muscular force, however, seems weak in proportion to the size of the muscles. All voluntary movements of the muscles are difficult for the patient to perform, owing to the stiffness present. Any attempt, after a rest period, to use a group of muscles causes a tonic contraction of these muscles, and it is impossible for him to relax these for a period of from ten to twenty seconds. All of the muscles are more or less involved, but the stiffness and tonicity following contraction are most marked in the hamstring muscles of the leg and in the muscles of the arm and forearm. When he clenches his first he is unable to open his hand for a period of several seconds. It is also difficult for him to start walking. All movements of the arm are done slowly and cause cramps which the patient refers to the region of the joints. He says that there is no pain in the muscles, only a sense of fatigue. There is some slight difficulty in opening his jaws, in protruding the tongue, and in opening the eyelids. The facial muscles are also slightly involved. The hand grip is weak. The interossei are but little affected, the patient being able to write quite well. The tonicity becomes most marked when the patient suddenly attempts to perform any energetic movement.

The muscles show the condition of exaggerated mechanical excitability characteristic of myotonia. Tapping the pectoral or other muscle groups produces a slow tonic contraction of the muscle, persisting for several seconds, and at the point of stimulation a tumor-like mass protrudes, giving rise to a localized bifurcation of the muscle from its origin to insertion.

The essential myotonic reaction can also be demonstrated in the altered electrical excitability. Stimulation of the muscle with a strong faradic current causes a prolonged tonic contraction. A weak faradic stimulation also gives rise to a prolonged contraction.

The examination of the blood showed only a secondary anemia, with red blood-cells, 4,300,000; white blood-cells, 7,000; hemoglobin (Sahli), 60 per cent. The differential count showed no material deviations from the normal. The routine examination of the urine was negative except for the presence of many triple phosphate cystals in the sediment. A Wassermann test and a tuberculin test both yielded negative results.

The patient left the hospital, Dec. 10, 1912, showing no material change in his condition from that found on admission.

III. REVIEW OF LITERATURE OF METABOLISM STUDIES IN VARIOUS NEUROMUSCULAR CONDITIONS

Rosenthal[2] found a lessened excretion of creatinin in a case of progressive muscular atrophy. Weiss,[3] in three cases of pseudohypertrophic muscular dystrophy, found a lessened creatinin excretion. Langer[4] also observed a lessened creatinin excretion in this disease, Jakubowitsch,[5] in two cases of pseudohypertrophic muscular dystrophy, found a low creatinin, urea, uric acid and chlorid excretion. MacLeod,[6] on the contrary, found no change in the metabolism of these substances in progressive muscular atrophy or in pseudohypertrophic paralysis.

The above-mentioned studies on creatinin excretion were carried out before the introduction of Folin's method for estimation of creatinin, and the old method of Neubauer, with or without Salkowski modification, was used for estimating creatinin. Another criticism of these results is that the patients were not on creatin-free or creatinin-free diets and the weight of the patient was not considered except in MacLeod's case.

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[2] Rosenthal: Handbuch der Diagnose und Therapie der Nervenkrankheiten, Erlangen, 1870.

[3] Weiss: Wien. med. Wchnschr., 1877, p. 701; 1883, p. 613.

[4] Langer: Deutsch. Arch. f. klin. Med., 1883, xxxii, 395.

[5] Jakubowitsch: Neurol. Centralbl., 1884, p. 279.

[6] MacLeod: Jour. Physiol., 1900, xxvi, Proc. Physiol. Soc., p. 7.

Spriggs,[7] however, in a case of pseudohypertrophic dystrophy, on a creatin-free diet, found the creatinin excretion to be about one-half the normal, and the percentage of nitrogen excreted as creatinin on an ordinary diet was also less than normal. Spriggs[8] also observed a decreased creatinin excretion in two cases of progressive muscular atrophy, in myasthenia gravis, and in amyotonia congenita.

In Spriggs' two cases of progressive muscular dystrophy the amount of creatinin in the urine was small and the diminution was proportional to the muscular wasting. In the case of amyotonia congenita the excretion of creatinin was also low, while in the case of myasthenia gravis the creatinin was diminished in lesser degree. He found that in all of these cases the excretion of uric acid was normal.

Bookman,[9] in a case of idiopathic osteosathyrosis with muscular atrophy and weakness, found in a six-day metabolism study that the patient had a positive balance of nitrogen (10.79 gm.), of sulphur (0.9 gm.) and of phosphorus (0.55 gm.), while a negative balance of magnesium oxid (0.183 gm.), and calcium oxid (4.94 gm.) was obtained. The creatinin coefficient was 6.5 mg. per kilogram weight and from 2.4 to 3 per cent. of the total nitrogen of the urine was excreted as creatinin-nitrogen. This amount of creatinin is considerably below the normal.

Mueller[10] found, in a case of progressive muscular atrophy, an increased excretion of sulphur and calcium, which he thought was due to a secondary atrophy of the bones. Pemberton,[11] in a case of myasthenia gravis found the creatinin excretion to be normal in amount but in relation to the percentage of total nitrogen excreted it was rather low (1.67 per cent.). He also observed a marked loss of calcium, for in a six-day period the patient had a negative calcium balance of 8.89 gm. with a positive nitrogen balance of 16.6 gm. Pemberton,[12] in a case of myotonia atrophica, found in a five-day period that the patient had a positive nitrogen balance of 28.26 gm. and a negative calcium balance of 0.566 gm. There was also a lessened amount of creatinin excreted in the urine.

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[7] Spriggs: Biochem. Jour., 1907, ii, 206.

[8] Spriggs: Quart. Jour. Med., 1907, i, 63.

[9] Bookman: THE ARCHIVES INT. MED., 1911, viii, 675.

[10] Mueller: Habilitätschr., Würzburg; abstr., Arch. f. Verdauungskr., 1897, iii, 282.

[11] Pemberton: Am. Jour. Med. Sc., 1910, cxxxix, 816.

[12] Pemberton: Am. Jour. Med. Sc., 1911, cxli, 253.

Diller and Rosenbloom[13] have studied the metabolism in a case of myasthenia gravis for a period of eight days. The nitrogen metabolism and urinary nitrogen partition, the sulphur metabolism and urinary sulphur partition, calcium and magnesium, phosphorus and fat metabolism was studied. The creatinin, uric acid and neutral sulphur excretions were decidedly less than normal. A considerable loss of calcium was found and the addition of 300 gm. of egg-yolk to the diet caused phosphorus retention, not accompanied by retention of either calcium or magnesium. The fat metabolism was normal.

Gittings and Pemberton[14] found no evidence of marked disturbance of calcium metabolism in a case of amyotonia congenita, in a boy of 21 months. They found a very low creatinin elimination, the average being daily 0.0166 gm., the creatinin coefficient 1.66 mg. per kilogram body-weight, while control cases of normal children showed a creatinin elimination of about 0.08 gm.

Findlay[15] in a case of myotonia found a creatinin coefficient which varied from 11 to 14 mg., which is rather high. The amount of creatinin excreted was from 1.8 to 2.3 gm. per day, while creatin was absent on a creatin-free diet.

IV. METABOLISM METHODS

During the entire experiment the patient received daily the Folin diet,[16] consisting of:

Whole milk	500 c.c.
Cream	300 c.c.
Eggs (whole)	450 gm.
Malted milk	200 gm.
Sugar	20 gm.
Sodium chlorid	6 gm.
Water, about	2,100 c.c. (distilled)

The mixture[17] was prepared fresh every day and portions taken out for analysis. The ammonia, total nitrogen, urea, creatin and creatinin were estimated in the fresh urine, the other constituents were estimated at the end of the experiment.

1. *Methods Used in Urine Analysis.*—The nitrogen was estimated according to Kjeldahl, the ammonia by Folin's[18] method, the total sul-

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[13] Diller and Rosenbloom: Am. Jour. Med. Sc., 1914 (in preparation). This paper also contains a detailed discussion as regards the influence of the internal secretory organs on the metabolism of calcium.

[14] Gittings and Pemberton: Am. Jour. Med. Sc., 1912, cxliv, 732.

[15] Findlay: Quart. Jour. Med., 1912, v, 495.

[16] Folin: Am. Jour. Physiol., 1905, xiii, 45.

[17] This diet was started November 20; the collection of excreta started November 22. The feces were marked off by means of carmin into periods of three days each.

[18] Folin: Ztschr. f. physiol. Chem., 1902, xxxvii, 161; Am. Jour. Physiol., 1903, viii, 330.

phur by Benedict's[19] method, total and ethereal sulphates by Folin's[20] method, the inorganic sulphates computed by subtracting the ethereal sulphates from the total sulphates and the neutral sulphur computed by subtracting the total sulphates from the total sulphur. Urea was estimated by Benedict's[21] method, total phosphorus by the Neumann[22] method, weighing the phosphorus as magnesium pyrophosphate, the total phosphates by titrating with uranium acetate solution, the earthy phosphates by titrating the precipitate produced by the addition of ammonia to a portion of the urine, with uranium acetate solution. Creatinin and creatin were estimated by Folin's[16] method, uric acid by the Kruger-Schmidt[23] method. Chlorids by the Volhard-Arnold method, calcium and magnesium by McCrudden's[24] method.

2. *Methods Used in Analysis of Food.*—Nitrogen by Kjeldahl method. Total sulphur by Wolff and Osterberg[25] modification of Benedict's method. Calcium and magnesium by McCrudden's method after ashing and extracting the ash with hydrochloric acid. Phosphorus by Neumann method. Fat was estimated in four-day periods by Soxhlet extraction.

3. *Methods Used in Analysis of Feces.*—Nitrogen by Kjeldahl method. Sulphur by oxidizing with fuming nitric acid followed by Benedict's method. Phosphorus by Neumann method. Calcium and magnesium by same method as used for food. Fat by the Kumagawa and Suto[26] method. The accompanying tables contain the data obtained in this study.

DISCUSSION OF TABLE 1

One of the great advantages in using the Folin diet for metabolism studies is the fact that the results found can be compared with the *normal* values that Folin obtained with this diet. In twelve days this patient lost 20.02 gm. of nitrogen, while the absorption of nitrogen and the urinary nitrogen portion (urea, ammonia, creatinin, uric acid and undetermined nitrogen) are perfectly normal in character.

One striking result found is the normal amount of creatinin excreted by this patient. One would think that if creatinin excretion depends on the muscular efficiency of an individual, as has been claimed by Shaffer,[27] our patient would show a lessened excretion of creatinin. It is to be remembered that the creatinin excretion is practically constant, on the same diet, from day to day, for each individual, although varying with the individual. It has been claimed that the main factor which influences the creatinin excretion is the

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[19] Benedict: Jour. Biol. Chem., 1909, vi, 363.

[20] Folin: Am. Jour. Physiol., 1905, xiii, 51; Jour. Biol. Chem., 1906, i, 131.

[21] Benedict: Jour. Biol. Chem., 1911, viii, 405.

[22] Neumann: Ztschr. f. physiol. Chem., 1902-03, xxxvii, 129; 1904-05, xliii, 35.

[23] Kruger-Schmidt: Ztschr. f. physiol. Chem., 1905, xlvi, 1; Jour. Biol. Chem., 1909, vii, 27.

[24] McCrudden: Jour. Biol. Chem., 1911, x, 187.

[25] Wolff and Osterberg: Biochem. Ztschr., 1910, xxix, 429.

[26] Kumagawa and Suto: Biochem. Ztschr., 1908, ix, 337.

[27] Shaffer: Am. Jour. Physiol., 1908-'09, xxiii, 1.

TABLE 1.—The Nitrogen Metabolism and Urinary Nitrogen Partition

Date	Urine*														Feces		Balance	
	Volume, c.c.	Total Nitrogen, gm.	Urea Nitrogen		Ammonia Nitrogen		Creatinin †			Uric Acid			Undetermined Nitrogen		Nitrogen		Nitrogen	
			Gm.	Pct. of Total Nitrogen	Gm.	Pct. of Total Nitrogen	Gm.	Nitrogen	Pct. of Total Nitrogen	Gm.	Nitrogen	Pct. of Total Nitrogen	Gm.	Pct. of Total Nitrogen	Gm.	Pct. of Nitrogen Intake	Intake	Balance
11/22	1940	15.0	12.9	86.0	0.60	4.0	1.42	0.53	4.8	0.38	0.13	0.87	0.84	5.6	....	....	15.8	
11/23	2000	14.6	12.7	86.9	0.58	3.9	1.40	0.52	4.0	0.34	0.11	0.75	0.69	4.7	1.98	12.5	15.8	—0.8
11/24	2390	16.4	13.8	84.2	0.84	5.1	1.61	0.60	3.7	0.40	0.13	0.79	1.03	6.3	1.98	12.2	16.2	—2.2
11/25	2270	14.0	12.3	87.8	0.56	4.0	1.45	0.54	3.8	0.38	0.13	0.93	0.47	3.3	1.98	12.5	15.8	—0.18
11/26	2280	16.6	14.0	84.3	0.86	5.2	1.56	0.58	3.5	0.40	0.13	0.78	1.03	6.2	1.60	10.4	16.6	—1.76
11/27	2210	16.8	14.2	84.5	0.88	5.2	1.55	0.58	3.4	0.38	0.13	0.77	1.02	6.1	1.60	10.4	16.7	—1.60
11/28	2215	16.9	14.4	85.2	0.88	5.2	1.56	0.58	3.4	0.38	0.13	0.77	0.91	5.4	1.90	12.1	15.6	—3.2
11/29	2360	17.2	14.5	84.3	0.89	5.1	1.57	0.58	3.4	0.42	0.14	0.81	1.10	6.4	1.90	12.5	15.2	—3.9
11/30	2330	19.1	15.6	81.6	0.96	5.0	1.61	0.60	3.1	0.46	0.16	0.84	1.78	9.3	1.90	9.9	16.0	—5.0
12/ 1	2050	16.4	13.9	84.8	0.85	5.1	1.12‡	0.41	2.5	0.42	0.14	0.85	1.10	6.7	1.82	11.6	15.7	—2.5
12/ 2	2200	16.6	14.1	84.9	0.87	5.2	1.62	0.60	3.6	0.42	0.14	0.84	0.90	5.4	1.82	8.3	22.0	+3.6
12/ 3	2390	17.1	14.7	85.9	0.82	4.8	1.50	0.56	3.2	0.48	0.16	0.94	0.60	3.5	1.82	8.8	20.6	+1.7
12/ 4	1700	17.3	14.9	86.1	0.84	4.8	1.60	0.59	3.4	0.48	0.16	0.93	0.80	4.6	1.82	12.2	14.9	—4.2

* No creatin was present in the urine except on December 3, after the addition of 300 gm. of egg-yolk on December 1 and 320 gm. on December 2. Dr. Rosenbloom hopes to study the influence of lecithin, kephalin and other substances containing methyl radicals on the excretion of creatin and creatinin.

† Average creatinin excretion per day = 1.5 gm.; average creatinin nitrogen excretion per day = 0.56 gm.; average creatinin nitrogen in per cent. of total nitrogen = 3.5 per cent.; average creatinin coefficient = 8.8 mg. nitrogen per kilogram body weight.

‡ On account of the low creatinin excretion on this date there is reason to think that we did not receive a complete twenty-four-hour specimen.

absolute amount of muscle tissue present in any individual, while the amount of muscular activity[28] is by itself without any effect. Therefore, we might conclude that the muscles in myotonia congenita are not degenerated and that the increase in size of the muscles may be a true hypertrophy, produced by the excessive work needed to overcome the spasm, as has been suggested by Knoblauch.[29]

Creatin was absent from the urine except on December 3, after the addition of 300 gm. of egg yolk on December 1 and 320 gm. on December 2.

It is possible that the methyl radicles present in the lecithin and kephalin of egg-yolk may have been the precursors of the creatin excreted on this day.

We would like to point out that in the present method for estimating creatin by converting it into creatinin and then considering the increased creatinin reading as due to creatinin formed from creatin, there are several sources of error. Some of these have been studied by Rose.[30] A more accurate method to show the presence of creatin would be to use some method which would allow the recognition of creatin as such.

The new diacetyl method of Walpole[31] would seem to fill this need, as it reacts to creatin and not to creatinin. One of us (R.) hopes to study by this new method the presence of creatin in the urine in acute fevers, exophthalmic goiter, muscular paralyses and during puerperium, while the uterus is involuting.

Creatin is not found in normal urines, on a creatin-free diet, and when endogenous creatin appears in the urine, some maintain that it is an evidence of a rapid loss of muscle-protein. According to this theory the absence of creatin in our case also speaks against there being any degenerative changes in the muscles in this disease.

DISCUSSION OF TABLE 2

The sulphur metabolism of this individual may be considered normal in character. In a period of ten days 0.9 gm. of sulphur was lost by our patient. The urinary sulphur partition (total sulphates, ethereal sulphates, inorganic sulphates and neutral sulphur) is normal.

DISCUSSION OF TABLES 3 AND 4

This patient in the metabolism period of ten days had a positive phosphorus balance of 1.5 gm., showing that during a time of nitrogen loss, phosphorus was retained. The amount of earthy phosphates and total phosphates may be considered normal in character.

A striking result in the study of the mineral metabolism of this case of myotonia congenita is the large loss of calcium from the body. In this metabolism study of ten days 4.09 gm. of calcium oxid and 0.56 gm. of magnesium

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[28] Shaffer: Am. Jour. Physiol., 1908, xxii, 445; Von Hoogenhuyze and Verploegh: Ztschr. f. physiol. Chem., 1905, xlvi, 415; Pekelharing and von Hoogenhuyze: Ztschr. f. physiol. Chem., 1910, lxiv, 262.

[29] Knoblauch: München. med. Wchnschr., 1908, lvii, 1560, 1903.

[30] Rose: Jour. Biol. Chem., 1912, xii, 73.

[31] Walpole: Jour. Physiol., 1911, xlii, 301.

TABLE 2.—THE SULPHUR METABOLISM AND URINARY PARTITION

Date	Urine									Feces		
	Sulphur	Sulphate Sulphur		Ethereal Sulphate Sulphur		Inorganic Sulphate Sulphur		Neutral Sulphur		Sulphur	Sulphur Intake	Sulphur Balance
	gm.	gm.	Pct.*	gm.	Pct.*	gm.	Pct.*	gm.	Pct.*	gm.	gm.	gm.
11/23	1.29	1.24	96.1	0.06	4.6	1.18	91.5	0.05	3.9	0.34	1.7	0.07+
11/24	1.49	1.40	93.9	0.22	14.8	1.18	79.1	0.09	6.1	0.34	1.7	0.13—
11/25	1.48	1.39	93.9	0.12	8.1	1.27	85.8	0.09	6.0	0.34	1.9	0.08+
11/26	1.58	1.51	95.6	0.16	10.1	1.42	85.5	0.07	4.4	....	1.9	.....
11/27	1.70	1.50	88.3	0.31	18.2	1.19	70.1	0.20	11.8	....	1.9	.....
11/28	1.70	1.56	91.8	0.21	12.4	1.35	79.4	0.14	8.2	0.37	2.0	0.07—
11/29	1.93	1.82	94.3	0.08	4.1	1.74	90.1	0.11	5.2	0.37	2.0	0.30—
11/30	1.92	1.80	93.8	0.47	24.4	1.33	69.4	0.12	6.3	0.37	2.2	0.09—
12/ 1	2.14	1.96	91.6	0.10	9.3	0.88	82.3	0.18	8.4	0.40	2.2	0.34—
12/ 2	2.00	1.85	92.5	0.23	15.3	1.12	74.6	0.15	7.5	0.40	2.4	0.0
12/ 3	1.97	1.85	93.9	0.21	12.4	1.38	80.6	0.12	6.1	0.40	2.4	0.03+
12/ 4	2.15	2.07	96.3	0.10	8.4	0.99	84.6	0.08	3.7	0.40	2.4	0.15—

* Total sulphur.

oxid was lost. Pemberton[11] found that his patient with myotonia atrophica lost in a five-day period 0.56 gm. of calcium. This loss of calcium occurred in spite of the large amount in excess of this element in the diet. Towle[32] found that 0.014 gm. of calcium oxid per kilogram of body weight is the daily requirement for this substance. Renvall[33] has found that an adult man required 0.98 gm. of calcium oxid per day. Our patient received on an average 2.87 gm. of calcium oxid per day or 0.045 gm. of calcium oxid per kilo. Therefore in our patient we have a condition whereby, although a large excess of calcium is taken in, for some reason he is not able to retain this substance. It was hoped that by feeding an extra amount of phosphorus a retention of calcium might be induced. Although the phosphorus was retained there was no improvement as regards the retention of calcium.[34]

TABLE 3.—The Calcium, Magnesium,—

	Urine							
Date	Total Phosphorus gm.	Earthy Phosphates as Phosphorus		Total Phosphates as Phosphorus		Chlorin	Calcium Oxid	Magnesium Oxid
		gm.	Pct.*	gm.	Pct.*	gm.	gm.	gm.
11/22	1.50	0.68	45.3	1.42	94.7	6.9	0.87	0.34
11/23	1.20	0.76	63.3	1.03	85.8	7.1	0.85	0.30
11/24	1.31	0.70	53.4	1.11	84.7	9.0	0.73	0.35
11/25	1.62	0.70	43.2	1.43	88.2	8.0	0.88	0.37
11/26	1.57	0.65	41.4	1.33	84.7	8.5	0.87	0.37
11/27	1.58	0.71	45.0	1.34	84.8	8.0	0.75	0.36
11/28	1.62	0.56	34.6	1.24	76.5	8.2	0.72	0.31
11/29	1.65	0.47	28.5	1.55	93.9	8.4	0.79	0.32
11/30	1.92	0.80	41.6	1.56	81.2	8.4	0.84	0.34
12/ 1	1.58	0.90	57.0	1.30	82.3	7.1	0.76	0.22
12/ 2	1.55	0.63	40.7	1.32	85.1	7.8	0.82	0.44
12/ 3	2.62	0.70	26.7	2.10	80.1	4.9	0.74	0.23
12/ 4	1.94	0.60	30.9	1.80	92.8	3.2	0.86	0.27

* Of total phosphorus.

It has long been known that the inorganic ions play a most important part in the normal action of muscle. May it not be that the disease "myotonia congenita" owes its origin to the fact that some abnormality is present preventing the normal metabolism of these substances, especially calcium? In favor of this hypothesis the work of Ringer and Sainsbury[35] may be cited. The so-called myotonic type of contraction of muscle found in myotonia congenita has been produced by Ringer and Sainsbury by transfusing the muscles of frogs with sodium phosphate after the motor end-plates were paralyzed by curare.[36] On the basis of these experiments it has been claimed that this disease is due to some fault in muscular metabolism, that the catabolism is at first increased and that there is some delay in the removal of waste products. May it not be that the calcium ion plays an important part in the removal of waste products arising during muscular action?

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[32] Towle: Am. Jour. Med. Sc., 1910. cxl, 100.

[33] Renvall: Skandin. Arch. f. Physiol., 1904, xvi, 94.

[34] After the completion of this work the communication of Dubois and Stolte (Jahrb. f. Kinderh., 1913, lxxvii, 21) was noted. They found that infants showed gains in the retention of calcium when the food yielded an excess of alkali or its equivalent of carbonate of sodium and potassium were added to the usual dietary. Dr. Rosenbloom hopes to study this point in adults.

[35] Ringer and Sainsbury: Brit. Med. Jour., 1884, ii, 114.

The disturbance in the calcium metabolism might also be explained on the basis of some lack in the function of certain internal secretory organs. McCrudden[37] found that improvements in the symptoms of osteomalacia, following castration, were accompanied by calcium retention, whereas there had been a loss before. Also, it has been found that injections of epinephrin[38] have been of value in osteomalacia and in rickets, which could be explained on the basis of the findings of Carnot and Slavu,[39] who found that, after subcutaneous injections of dogs with epinephrin, ossification was much greater than in controls.

—Phosphorus and Chlorin Metabolism

Feces			Intake			Balance		
Calcium Oxid	Phosphorus	Magnesium Oxid	Calcium Oxid	Magnesium Oxid	Phosphorus	Calcium Oxid	Magnesium Oxid	Phosphorus
gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
...	....	....	2.95	0.35	2.6	......	......	
2.4	1.1	0.10	2.90	0.35	2.6	—0.35	—0.05	+0.3
2.4	1.1	0.10	2.86	0.36	2.6	—0.27	—0.09	+0.18
2.4	1.1	0.10	2.80	0.36	2.8	—0.48	—0.11	+0.08
...	....	....	2.92	0.31	2.8	......	......	
...	....	....	2.96	0.31	2.8	......	......	
2.3	1.3	0.11	2.90	0.32	2.4	—0.12	—0.10	—0.52
2.3	1.3	0.11	2.94	0.32	2.4	—0.15	—0.11	—0.55
2.3	1.3	0.11	2.82	0.40	2.4	—0.32	—0.05	—0.82
2.6	1.5	0.13	2.82	0.40	2.4	—0.54	+0.05	—0.68
2.6	1.5	0.13	2.64	0.42	5.2	—0.78	—0.15	+2.15
2.6	1.5	0.13	2.92	0.42	5.1	—0.42	+0.06	+1.0
2.6	1.5	0.13	2.80	0.39	2.3	—0.66	—0.01	—1.14

V. SUMMARY

1. In a metabolism experiment of thirteen days' duration on a case of myotonia congenita, we have found that the patient lost 20.02 gm. of nitrogen, while the urinary nitrogen partition (urea, ammonia,

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[36] Panzini (Semaine Méd., 1908, p. 175) claims that the muscles of newborn mammals, the normal muscles of the toad and muscles poison by veratrin, digitalin, hellebore, oxalic acid and sodium salts, gives a myotonic reaction.

[37] McCrudden: Am. Jour. Physiol., 1905, xiv, 211; 1906, xvii, 211.

[38] Bossi: Centralbl. f. Gynäk, 1907, xxxi, 1560; Stocker: Schweiz. Corr-Bl. f. Aerzte, 1909, xxxix, 433.

[39] Carnot and Slavu: Compt. rend. Soc. de biol., 1910, xlviii, 832.

creatinin, uric acid and undetermined nitrogen) was normal in character. Creatin was absent from the urine except after the administration of 620 gm. of egg-yolk.

2. In a period of ten days 0.9 gm. of sulphur was lost, while the urinary sulphur partition (total sulphates, ethereal sulphates, inorganic sulphates and neutral sulphur) is normal in character.

3. In a period of ten days the patient had a positive phosphorus balance of 1.5 gm., showing that during a time of nitrogen loss, phosphorus was retained. The amount of earthy phosphates and total phosphates in the urine may be considered normal.

TABLE 4.—The Fat Metabolism

Period	Intake of Fat gm.	Fat in Feces gm.	Percentage Percentage Absorbed
11/23–11/26	560.8	55.5	90.1
11/27–11/30	548.5	49.9	90.9
12/ 1–12/ 4	745.0	76.2	89.8

Table 4 shows the fat metabolism to be normal in character, with an absorption of about 90 or 91 per cent. of the ingested fat.

TABLE 5.—Summary of Metabolism Data

Substance	Intake gm.	Output gm.	Balance gm.
Nitrogen*	201.1	221.12	20.02—
Sulphur†	20.90	21.80	0.90—
Phosphorus†	30.20	28.70	1.50+
Calcium oxid†	28.40	32.49	4.09—
Magnesium oxid†	3.74	4.30	0.56—
Fat*	1854.3	181.6	1672.7 +

* Data for period of twelve days.
† Data for period of ten days.

4. In ten days 4.09 gm. of calcium oxid and 0.56 gm. of magnesium oxid were lost by this patient. A retention of phosphorus produced by feeding egg-yolk did not cause a retention of calcium.

5. Evidence is produced to show that the loss of calcium may play a part in the production of the symptoms of myotonia congenita.

6. The fat metabolism was normal in character, with an absorption of about 90 or 91 per cent. of the ingested fat.

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STUDY OF A CASE OF APLASTIC ANEMIA *

JOHN H. MUSSER, JR., M.D.

PHILADELPHIA

By the term "aplastic anemia" is understood a type of anemia differing in many respects from ordinary types of primary or secondary anemia but marked especially by retrogressive changes in the bone-marrow which result in a change of the normal red marrow to a fatty marrow. The term was first used by Ehrlich[1] in 1888, in connection with an anemia of this type following severe uterine hemorrhage.

Isolated examples of aplastic anemia have since been reported and in 1908 Cabot[2] collected reports of 24 authentic cases and discarded 11 other cases as incomplete or indefinite. In 1911 Hirschfeld[3] in a discussion of the pathogenesis of the malady, gives briefly the essential features of 44 cases, of which 3 had been rejected and 12 accepted by Cabot. In the literature of the subject I have found 3 other cases, reported respectively by Crummer,[4] by Stitt,[5] and by Whipple[6] in the course of a paper on a different subject. These 59 authenticated cases probably do not by any means represent all the cases of aplastic anemia that have occurred in the past twenty-six years, but merely those, for the most part, that include a necropsy. Post-mortem examination of the bone-marrow is usually considered essential to an exact diagnosis. I believe, however, that the clinical course, the blood-findings and symptomatology in general are so typical and so unmistakable that the diagnosis can be readily made during the life of the patient, and I therefore have no hesitancy in reporting the following case, although it lacks the final evidence acquired only by the post-mortem examination. In connection with this report I present first a brief summary of our knowledge of aplastic anemia.

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* Submitted for publication, May 21, 1914.

[1] Ehrlich, P.: Ueber einen Fall von Anämie mit Bemerkungen über regenerative Veränderungen des Knochenmarkes, Charité-Ann., 1888, xiii, 800.

[2] Cabot, R. C.: Diseases of the Blood, Osler's Modern Medicine, Philadelphia, Lea & Febiger, 1908, iv, 637.

[3] Hirschfeld, H.: Folia haematol., 1911, xii, 347.

[4] Crummer, LeRoy: A Case of Aplastic Anemia, Jour. Am. Med. Assn., 1907, lix, 2086.

[5] Stitt, E. R.: Aplastic Pernicious Anemia Associated with Agchylostomiasis, U. S. Nav. Med. Bull., 1911, v, 345.

[6] Whipple, G. H.: Hemorrhagic Diseases: Antithrombin and Prothrombin Factors, The Archives Int. Med., 1913, xiii, 649.

GENERAL SUMMARY OF THE SUBJECT

Etiology.—In Cabot's series the great majority of persons were under the age of 34, and in the additional cases that have been studied the same holds true: the disease is essentially one of early adult life. The extremes are respectively 5[7] and 68[8] years.[9] The ratio of the two sexes in 35 cases was males 20 to females 15.

Clinical Course.—The course of the disease is rapid and progressive. Dalton[10] reports a case of a few days' duration and Chauffard,[11] the authenticity of whose figures may well be doubted, one of six years' duration. The average course, however, is rarely more than a few weeks to one or two months, with rapid progression to a fatal termination. Larrabee's case[7] is the only one in which a frank remission occurred. In addition to the usual features of a progressive severe anemia, extremely characteristic is the tendency to subcutaneous hemorrhages and hemorrhages from mucous membranes. In some cases[12, 13, 14, 15] it seems quite possible that the sudden appearance of hemorrhage in an apparently healthy person, was the first manifestation of the disorder. Another symptom more common than in the usual types of anemia, is fever, which often reaches a high point some time or other during the course of the disease.

Blood-Picture.—Most typical and characteristic is the blood-picture —a picture which is seen in no other blood disorder. Usually, when the condition is first recognized, a severe and intense anemia of the hemoglobin and red cells has already developed. The relation of the one to the other varies so that the index is claimed by some authors to be high,[16] while others[17, 2] find a low color-index. The low color-index would seem to be the correct observation, because a high color-index is due either to megalocytes or erythroblasts in the peripheral blood, both of which cells are absent from the peripheral circulation in the true types of aplastic anemias. The high color-index cases might well represent cases which have developed from the typical hyperchrome pernicious anemias, and show a tendency on the part of the marrow to regeneration of the megaloblastic type. Naegeli[18] and M'Weeney[19] have pointed out the fact that a high color-index is indicative of blood regeneration.

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[7] Larrabee, R. C.: Aplastic Anemia, with Report of a Case, Am. Jour. Med. Sc., 1911, cxlii, 57.

[8] Kurpjuweit, O.: Ueber letale Anamien im Greisenalter, Deutsch. Arch. f. klin. Med., 1905, lxxxii, 423.

[9] Authenticity of the case dubious.

[10] Dalton, N.: Two Cases of Malignant Anemia, without Signs of Erythrocytolysis and Associated with Suppuration About the Jaws, Tr. Clin. Soc., London, 1904, xxxvii, 101.

[11] Chauffard, M.: Un cas d'anémie pernicieuse aplastique, Bull. Soc. méd. d'hôp., 1904, xxi, 313.

[12] Blumenthal, R.: Pathogénésie d'anémie aplastique, Arch. d. mal. du cœur, 1907, v, 184

[13] Hirschfeld, H.: Ueber schwere Anämien ohne Regeneration des Knochenmarks, Berl. klin. Wchnschr., 1906, xliii, 546.

[14] Muir, R.: A Case of Purpura and Intense Anemia with Marked Deficiency in the Red Bone-Marrow, Brit. Med. Jour., 1900, ii, 909.

[15] Steinhaus, I., and Stordeur, L.: L' anémie dite aplastique, Arch de méd. expér. et d'anat. path., 1908, xx, 805.

[16] Carslaw, J., and Dunn, S.: Case of Aplastic Anemia, Glasgow Med. Jour., 1910, lxxiii, 331.

[17] Lavenson, R.: The Nature of Aplastic Anemia and Its Relation to Other Anemias, Am. Jour. Med. Sc., 1907, cxxxiii, 100.

There is also a pronounced leukopenia. In one of Selling's[20] cases of benzol (benzene) poisoning with an aplastic picture, the leukocytes dropped to 140 per cubic millimeter before death, and in Krantz's case[21] of bothriocephalus anemia with aplastic marrow, the total count was 800 per cubic millimeter. These figures represent extremes, but the counts in most cases rarely exceed 2,000 per cubic millimeter. The study of the morphology of the stained blood shows that the red cells are apparently normal in every respect. There is no poikilocytosis and no anisocytosis: the cells are normal in size with possibly a slight tendency to macrocytosis. Polychromatophilia and stippling of the red cells is absent. These observations are of interest, showing, as they do, that in anemias in which the bone-marrow is non-regenerative, there is no tendency to abnormal forms. In other words, there is definite evidence that red blood-cells, altered in shape and form probably represent immature, rapidly formed cells, while polychromatophilia is found in cells which are not degenerative.[22] Erythroblast cells of all types are practically entirely wanting and in extremely rare cases only are they found in the circulating blood. The differential leukocyte count readily accounts for the leukopenia. There is an absolute decrease in the number of granular leukocytes, the polynuclear forms constitute but from 8 to 20 per cent. of all the forms and there is a relative increase in the percentage of lymphocytes, but no change in the actual number of these cells per cubic millimeter. The granular cells are almost absent, while not only the non-granular cells are not decreased but also in some cases there is an absolute as well as relative increase.[23, 24]

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[18] Naegeli, O.: Blutkrankheiten und Blutdiagnostik, Leipsic, von Veit & Co., 1912, p. 136.

[19] M'Weeney, E. J.: A Case of Acute Aplastic Anemia, Jour. Path. and Bacteriol., 1909, xiv, 142.

[20] Selling, L.: Benzol as a Leukotoxin, Johns Hopkins Hospital Monographs, 1913, new series, ii.

[21] Krantz, E.: Ueber Bothriozephalusanämie mit aplastichen Knochenmark, Inaug. Diss., Zurich, 1906, quoted in Folia haematol., 1907, iv, supp. bd., p. 245.

[22] Naegeli, O.: Blutkrankheiten und Blutdiagnostik, Leipsic, von Veit & Co., 1912, p. 144.

This finding may be considered as contributing splendid evidence in favor of the polyphyletic theory of the origin of the blood-cells upheld by Blumenthal, Ehrlich, Morawitz, Naegeli, Sternberg,[25] etc. These authors claim that there are separate parent-cells for the myeloid and the lymphatic-cell series. The first, arising from the blood-vessel endothelium, gives rise to the myeloblast and erythroblast cells, ultimately becoming the granulocyte and forming the red cells; the latter series, arising from the lymph-vessel endothelium, gives rise to the lymphoblast and eventually the lymphocyte. The defenders of the monophyletic theory (Arnold, Grawitz, Dominici, Maximow, Pappenheim, etc.) contend that all blood-cells arise from a common mother-cell, the large lymphocyte or lymphoidocyte.

If the lymphocytes arise anywhere in the hemopoietic system it would seem natural to suppose that their decrease would correspond with that of the other cells when the marrow, the chief source of all blood-cells, is functionless. If, however, they arise from a separate mother-cell whose origin is in the lymph-glands, it is entirely correct to assume that they would be unchanged in the blood-picture of an anemia, the chief characteristic of which is the marrow aplasia. Very similarly might the absence of change in number of the lymphocytes be considered an argument in favor of the theory that these cells are developed almost entirely in the lymphoid structures.

At necropsy the most characteristic finding is a complete and total aplasia of the bone-marrow of the long bones. There is an absence of erythroblastic tissue so that the normal red marrow is replaced by fat in which few, if any, cellular elements are discoverable. The spleen is of the hyperplastic type seen in pernicious anemia. There is in a few of the reported cases a definite hemosiderosis, but in the majority this finding is not reported.

REPORT OF CASE

The following case was seen in the service of Dr. Sailer at the Presbyterian Hospital while I was acting as his assistant and I am indebted to Dr. Sailer for the opportunity of studying it and for permission to report it.

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[23] Larrabee's case (see note 12) showed 4.567 per cubic millimeter; Thomas and Rolleston's case (see note 24) 60,460 per cubic millimeter; Muir's case (see note 15) 4.900 per cubic millimeter.

[24] Thomas, W., and Rolleston, H. D.: Fatal Aplastic Anemia, Brit. Med. Jour., 1910, i, 16.

[25] Gruner, O. C.: Biology of the Blood-Cells, New York, William Wood & Co., 1914, p. 30.

History.—Linda R., aged 47, American, white, was admitted Jan. 27, 1914, and discharged January 30. The principal complaint was of great weakness and fever.

Present Illness.—Dec. 1, 1913, the patient was told that she looked pale and sick. Between then and December 20 she noticed that she was gradually getting weaker. On December 20 she became so weak that she had to stop work for a few days. About this time she had great difficulty in keeping warm and her feet were continually cold. December 24 she worked for half a day, but then had to go to bed on account of weakness. She worked from January 1 to January 9, and again had to stop work, but occupied herself around the house until Jan. 25, 1914, when she had to go to bed on account of extreme weakness and the appearance of fever. Patient had had no chills, sweats or pains. Once or twice she expectorated dark blood and blew dark blood from her nose. She has noticed in the past few weeks that the slightest knock will cause a large bruise which quickly disappears. Has a poor appetite, but no indigestion and bowels move regularly. For the last two weeks she has noticed edema of ankles and dyspnea on exertion. No frequency of urination; no cough.

Previous History.—In childhood the patient had "intermittent fever"; is subject to tonsillitis; claims her husband infected her with lues when she was 26 years old; was treated for one year. One child was born which lived for three days. No miscarriages. Health otherwise always splendid. Had been a cashier in subway station for past three years. Eating habits are good, but drinks coffee to excess. Sleeps in a well-ventilated room. Moderate drinker of beer and occasionally whisky. Menses normal in every respect.

Family History.—Father and mother living and well. Does not live with husband. Four brothers and sisters living and well.

Physical Examination.—Patient is apparently about 35 years old; large stature, well developed, rather fat and lying comfortably in dorsal decubitis. Skin is soft, moist and well nourished, but sallow. Eyes are normal, pupils equal and react to light and accommodation. Conjunctiva and mucous membranes pale, particularly in mouth. Teeth and gums in splendid condition. Tongue slightly coated and tremulous. No general adenopathy. No thyroid enlargement or abnormal neck pulsations.

Thorax.—Large chest with good, free and symmetrical expansion, costoabdominal type of breathing. There are no abnormal pulsations or thrills. Lungs exhibit normal percussion, fremitus and breath-sounds throughout. Heart borders: third rib, right border of sternum, fifth space, mid clavicular line. At the apex is heard a soft systolic murmur unmodified by breathing, which is not transmitted into axilla or over precordium. Pulse weak, rapid and of poor volume and tension.

Abdomen.—Large, fat and everywhere soft. No palpable organs, no masses, no tenderness. Liver and spleen on percussion are not enlarged.

Extremities.—On left arm is a bruise 3 cm. in diameter with several small points of subcutaneous hemorrhage about it; similar spots on leg. On the inner side of the thigh is a small ulcer surrounded by minute purpuric spots which are also present on opposite leg. On the arm, following the use of the blood-pressure cuff, there developed a band of subcutaneous hemorrhagic extravasation exactly limited by the borders of the cuff. Patellar reflexes normal. No Babinski.

When admitted, patient's temperature was 103.2 F., pulse 120, respiration 24. Blood-pressure: systolic, 132; diastolic, 75. Erythrocytes, 1,390,000. Hemoglobin, 21 per cent. Leukocytes, 1,950. Differential count of leukocytes: large and small lymphocytes, 89 per cent.; polymorphonuclear neutrophils, 11 per cent. Urine examination: specific gravity, 1.020; acid; albumin, trace; sugar, none; many white blood-cells; no casts; trace of bile.

Examination by post-vital staining showed in 3,000 erythrocytes no skeined forms. Bleeding time, twenty minutes. Coagulation time, fourteen minutes (Dorrance coagulometer).

Resistance of erythrocytes to hypotonic salt solution:

0.25 to 0.375 = Complete hemolysis
0.40 = Almost complete hemolysis
0.425 = Almost complete hemolysis
0.450 = Fair hemolysis
0.475 = Very slight hemolysis
0.50 to 0.65 = No hemolysis

Blood-culture negative; blood for Wassermann test taken, but through some error was misplaced.

Treatment and Course.—Temperature, pulse-rate and respiration remained practically unchanged the first forty-eight hours in hospital. During this time patient was apparently comfortable, entirely rational but very sluggish and willing to sleep all the time. During the last twelve hours before death the temperature gradually fell to 100.2; while the pulse-rate increased to 136 and the respiration to 36. January 29, second day after admission, patient passed some blood mixed with the feces. This was repeated in the next twenty-four hours and twice in addition there was a passage of about 600 c.c. of dark clotted blood, the last several hours before death, which occurred at 9:05 a. m., January 30. The treatment was supporting and stimulating. Horse-serum, 20 c.c. was given after the first hemorrhage and calcium lactate was given in gram doses four times a day immediately after admission.

DISCUSSION OF THE CASE

This case presented so typically all the findings of aplastic anemia that we had no hesitancy in making that diagnosis. The etiology was obscure. So far as we could determine, the patient had been in good health up to the time the anemia was first noted. From then on the course was rapidly downward and in a short time terminated in death. The only possible etiologic factor was syphilis, but there was no definite proof that the patient had ever been infected; certainly there was no gross evidence of activity of a syphilitic process. A septicemia might be thought of, but there was no primary focus; the temperature was markedly elevated but very regular; the blood-culture was negative. In differentiating the disorder some type of purpura hemorrhagica might have been diagnosed but in this case the purpura was undoubtedly a symptom, certainly not a disease *sui generis,* if it ever is; neither was there an antecedent anemia. This of course, could not be determined absolutely, but in view of the splendid previous health of the patient it is safe to presume that she had not been a sufferer from pernicious anemia. These conditions are the only ones which might confuse the diagnosis; but so typical was the blood-picture that the only sequence of events that might have occurred would have resulted from the secondary disablement of the bone-marrow by some toxicosis.

In the study of the blood three observations about which very little has been written in this connection were made:

1. The coagulation time and the bleeding-time were both considerably delayed, though in Whipple's case[6] only the bleeding-time was prolonged.

2. I could find, employing the same technic with which I have been working in connection with another problem, no evidence of reticulated or skein-forms of red cells. The total absence of these cells would be expected, however, and would tend to show that the conception of Ferrata,[26] Hertz[27] and others that these cells are indicative of normal regeneration and are presumably young or unripe form of red cells, is correct.[22]

3. In observing the resistance of the red cells to hypotonic salt solution, it was found that the maximal resistance was decreased while the minimal resistance was unchanged. This may be interpreted as showing that there was almost a total absence of the younger cells, the most resistant of all cells, while the old cells in every respect are normal and, not acted on by hemolysins, preserved the same ability that they normally possess to resist various strengths of hypotonic salt solution.

The experimental evidence of the first portion of this surmise is found in a recent work of Handrick,[28] who employed saponin to produce artificial anemia. He found that this poison, whose action is largely on the marrow, caused a decrease in the maximal resistance of the red cells while the minimal resistance was lessened. In other words, in the myelopathic type of anemia, in which there is nonfunctionary marrow and consequently an absence of young forms, there develops a decrease in the maximal resistance. That it also causes a decrease in the minimal resistance is, however, dubious. Handrick used saponin in such strengths that the poison, while throwing the marrow out of commission also acted as a hemolysin. The rapidity with which the blood-counts fell—a high-grade anemia was evident in two days—could be explained only by the hemolysis of the red cells. This cannot be explained by a myelopathy decrease. As shown by Zoja,[29] the life of the erythrocyte is ten days. Failure of regeneration of the marrow for two days would only cause a reduction of the cells equivalent to one-fifth of the total number, an anemia much slighter than that Handrick obtained. It is therefore fair to assume that the diminished minimal resistance is due to the increased fragility of the red cells that occur in any hemolytic anemia

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26. Ferrata, A.: Ueber die klinische und morphologische Bedeutung der vitalfärbbern Substanz und die basophile Punktierung der Erythrozyten, Folia haematol., 1910, ix, 253.

27. Hertz, R.: Hématies granuleuses et ponctuées, Arch. d. mal. du cœur, 1913, v, 29.

28. Handrick, E.: Ueber die Bëeinflussung der Resistenz der roten Blutkörperchen durch hämatoxische Substanzen, Deutsch. Arch. f. klin. Med., 1912, cvii, 312.

29. Zoja, quoted by Gruner: Biology of the Blood-Cells, New York, William Wood & Co., 1914, p. 73.

of acute character before there is the compensatory increased resistance that is set up by hemolytic substances sooner or later.

Morawitz and Pratt,[30] Itami and Pratt,[31] and Rosenthal[32] have shown with phenylhydrazin, benzidin and blood poisons that the minimal and the maximal resistance, particularly the latter, is much increased: but these observations are on animals poisoned by long-continued administration of toxic substances with resultant active regeneration of the red cells. This increased resistance of the erythrocytes of experimentally induced anemia of animals, as shown by Snapper,[33] is an evidence of the presence of young erythrocytes.

PATHOGENESIS

In considering the pathogenesis of aplastic anemia it should be borne in mind that, considering their genesis, there are three main groups of anemias: (1) the type that occurs as a result of hemorrhage, acute or repeated; (2) the type that depends on primary disease of the marrow and is exemplified by the anemia that occurs in the course of leukemia or of osteosclerosis; (3) the type of anemia which depends on a direct or indirect destructive action of some toxin on the red cells, in which category are included the ordinary types of anemias secondary to some evident cause (not hemorrhage, however), the phanerogenetic anemias and the so-called primary, pernicious, idiopathic, Addisonian or "Biermerschen" anemia. The classification of aplastic anemia has evoked extensive discussion, which has been, for the most part, whether it should be considered as coming under one of the main varieties or as a subvariety of pernicious or secondary anemia. Gulland and Goodall[34] claim to have seen aplastic anemia when death has followed repeated or continuous hemorrhage, when it was due to a severe toxin of unknown cause acting on a marrow inherently weak, and when it occurred as a last stage of pernicious anemia.

Hirschfeld,[3] in discussing the pathogenesis of aplastic anemia, says that it is considered by Pappenheim and von Grawitz as a type of idiopathic pernicious anemia which runs a non-regenerative course; by

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30. Morawitz, P., and Pratt, J.: Einige Beobachtungen bei experimentellen Anämien, München. med. Wchnschr., 1908, lv, 1817.

31. Itami, S., and Pratt, J.: Ueber Veränderungen der Resistenz und der Stromata roten Blutkörperchen bei experimentellen Anämien, Biochem. Ztschr., 1909, xviii, 302.

32. Rosenthal, R. F.: Die sogenannte Pachydermie der Erythrozyten bei Phenylhydrazinanämie, Folia haematol., 1910, x, 253.

33. Snapper, J.: Einfluss des Auswaschens auf der Resistenz dez roten Blutkörperchen, Biochem. Ztschr., 1912, xxiii, 266.

34. Gulland and Goodall: The Blood, New York, E. B. Treat & Co., 1912, p. 155.

Naegeli as a special type of pernicious anemia; by Ziegler as the severest form of anemia; while Helly and Bloch assert that it depends on primary weakness of the bone-marrow.

Türk[35] divides anemias into four etiologic classes and considers aplastic anemia as a subtype of chronic hemolytic anemia.

Deganello[36] believes, with the French school, that aplastic anemia is provoked by an intense blood destruction, accompanied by *anhématopoiese.*

Lavenson[37] in a paper read before the Association of American Physicians in 1906 concludes that aplastic anemia is always a form of pernicious anemia. In the discussion Stengel agreed with that opinion, while Thayer felt that there is a sharp distinction between the two types.

Cabot[38] believes that the disorder differs in degree only from the ordinary pernicious anemia, while Carslaw and Dunn[17] believe that the disorder is not a specific disease but depends on a number of causes; the failure of the marrow to regenerate depends either on excessive action of the toxin or on individual susceptibility.

Blumenthal[39] considers aplastic anemia the last stage in the series of the anemias and that it is generally secondary to some incessant hemorrhage.

Sufficient authorities are quoted to show that aplastic anemia is considered to arise (1) as secondary to repeated hemorrhage, or (2) as secondary to the destructive action of some toxin on the erythrocytes without the action being hindered by an adequate regenerative process of the marrow, or (3) as a type of pernicious anemia. The opinion is certainly not extensively held that the disorder is due to a primary disturbance of the bone-marrow induced by a toxin which spends itself largely on the marrow, but such a condition is quite possible and, in the light of recent hematologic work, in many ways seems to be more probable than the theories which attribute the condition to a primary loss or destruction of the red cells.

The view first advanced by Ehrlich[1] and recently reviewed by Blumenthal[13] that the aplasia is the result of failure of the bone-marrow to react to the strain thrown on it by repeated hemorrhage, seems to be controverted by the experimental work of Blumenthal

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35. Türk, W.: Quoted by A. Pappenheim, Folia haematol., 1913, xiii (Zentral-organ), p. 275.

36. Deganello: Purpura hemorrhagique et anémie aplastique aiguë, Gazz. d. osp., July 4, 1909, quoted in Arch. d. mal. cœur, 1910, iii, 760.

37. Lavenson, R.: Aplastic Anemia, Tr. Assn. Am. Phys., 1906, xxi, 307.

38. Cabot, R. C.: Osler's System, Philadelphia, Lea & Febiger, iv, 611.

39. Blumenthal, R.: Ueber aplastische Anämie, Deutsch. Arch. f. klin. Med., 1907, xc, 132.

himself working with Morawitz.[40] These authors produced an anemia by frequently repeated bleedings, causing a lowering of the hemoglobin and red-cell count but having practically no effect on the leukocyte count except perhaps to increase the total number of cells per cubic millimeter, and the percentage of polynuclears. They produced bone-marrow changes which reminded them somewhat of aplastic marrow; degenerative changes of the marrow were produced but the typical aplastic yellow fat marrow was not. These authors themselves say that the blood-picture cannot be looked on as one of typical anemia on account of the leukocyte picture and that such a result might be achieved perhaps if the bleeding were continued for a long time—a consideration which is contrary to the onset and course of the disease clinically. Experimentally, then, evidence is lacking that repeated hemorrhage causes aplastic anemia; while in those cases that have been reported clinically as aplastic anemias following hemorrhage, I have no doubt that the hemorrhage did not initiate the disorder but was merely the first of those symptomatic hemorrhages that occur during the course of practically all the reported cases, and which were so severe in the case here reported.

To compare aplastic anemia to pernicious anemia it will be necessary first to discuss the theories of the genesis of this type of anemia. At one time the idea was wide-spread that the disease was due to a primary disorder of the bone-marrow, either as a functional asthenia or as the result of some toxin acting on the marrow specifically. These views, based on the hypothesis that changes in the marrow result in the liberation of immature cells which are more readily destroyed than the normal cells, causing extensive hemosiderosis, have been very generally discarded, particularly since more recent hematologic work has shown that the young erythrocytes are the most resistant. In place of these theories, the view that pernicious anemia is the result of primary hemotoxicosis, the toxic agent affecting the blood alone or the marrow also in part, has gained great credence; or as Naegeli[22] expresses it, pernicious anemia is a very constant toxigenetic anemia, brought on by toxins from various sources.

These toxins may act in three ways: (1) as hemolytic agents acting directly on the red cells in the general circulation (or in the portal circulation — Addisonian anemia);[41] (2) as agents causing increased vulnerability of the red cells which are consequently more readily destroyed by the hemolytic tissues; (3) as stimulants to the hemolytic tissues causing a hyperhemolytic action on their part.

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[40] Blumenthal, R., and Morawitz, P.: Experimentelle Untersuchungen über post-hämorrhagische Anämien und ihre Beziehungen zur aplastischen Anämien, Deutsch. Arch. f. klin. Med., 1907, xcii, 25.

[41] Hunter, W. K.: Recent Advances in Hematology, New York, William Wood & Co., 1912, p. 66.

The first view has been discredited by the work of M'Neil,[42] who has shown that the blood-serums in cases of pernicious anemia do not hemolyze washed corpuscles of normal individuals. Either of the latter two theories may be correct.

It has been shown that the view that pernicious anemia depends on excessive hemolysis of the red cells, no matter how produced, is essentially correct or at least is generally conceded. Now if we expect to prove that aplastic anemia is merely a variety or subgroup of pernicious anemia in which the bone-marrow has failed to respond to the unusual calls made on it by excessive hemolysis, it will be necessary to show that such hemolysis has taken place.

In considering the *intra-vitam* evidence we know that we are dealing with a disorder that is essentially acute and rapid in its course. A hemolysin that will produce such a rapidly developing anemia must naturally cause evidence of excessive and rapid hemolysis, of which we should expect to get some clinical manifestation. Such, however, is not the case. There is no evidence of urobilogenuria nor of jaundice, nor is the resistance of the erythrocytes to hypotonic salt solution decreased as we find it is experimentally when hemolytic immune serum is injected into animals.[43] In cases of acute or exaggerated hemolytic anemias, so-called, the course of the disease is frequently rapid; intermissions may occur; the blood-picture is similar to that of aplastic anemia only in the anemia of the red cells and the hemoglobin, but otherwise varies markedly.

These differences are well exemplified in a recent article by Garsböck,[44] who reports three cases of hemolytic anemia. The clinical differences between aplastic anemia and these cases of acute hemolytic anemia is most striking. In these cases there was found postmortem evidence of wide-spread hemolysis throughout the tissues while the marrow was a dark red hyperfunctioning marrow, findings directly opposed to those found in aplastic anemia. Similarly, experimentally acute hemolytic anemias produced in animals show at necropsy a red normoblastic marrow which has responded promptly to the sudden call made on it.[45]

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[42] M'Neil, C.: The Resistance of Human Red Corpuscles in Health and Disease to Hemolysis by Saponin, Jour. Path. and Bacteriol., 1910, xv, 56.

[43] Karsner, H. T., and Pearce, R. M.: A Study, by the Methods of Immunology, of the Increased Resistance of the Red Blood-Corpuscles After Splenectomy, Jour. Exper. Med., 1912, xvi, 769.

[44] Garsböck, F.: Beitrag zur Klinik hämolytischer Anämien mit herabgesetzter osmotischen Erythrocytenresistenz, Deutsch. Arch. f. klin. Med., 1913, cx, 413.

[45] Buting, C. H.: Experimental Anemias in Rabbits, Jour. Exper. Med., 1906, viii, 625.

If the relationship of pernicious anemia, a type of chronic hemolytic anemia, to aplastic anemia be considered from the clinical point of view, it must be conceded that there is no similarity whatsoever between them in the course of the disease, in the blood-picture or in the other findings. This difference is explained on the basis of failure of the marrow to react to the usual demand on it, consequently with the rapidly developing anemia, with the variation in the resistance of the erythrocytes, the absence of the erythroblasts, and so on. The tendency to subcutaneous and other hemorrhages is considered a peculiar and marked evidence of hemolysis. If the marrow causes these changes and results in the dissimilarity between aplastic and pernicious anemia, then evidence must be sought in the post-mortem changes in order to establish adequate relationship between the two. One of the most characteristic changes found in those dead of pernicious anemia is the intense hemosiderosis of the liver, spleen and lymph-glands. Except in a few cases, for example, those reported by Lavenson,[17] Deganello,[36] Stone,[46] and Muir,[15] there is almost a total absence of any report of these findings. Moreover, the cases mentioned above were all accompanied by extensive hemorrhage into the tissues, sufficient cause for the presence of iron pigment. In addition, according to the post-mortem reports, there is very little evidence of excessive hemolysis in the spleen and hemolymph glands such as occurs in pernicious anemia. The bone-marrow differences are also marked, the fat aplastic marrow on the one hand contrasted with the rich red megaloblastic marrow on the other. The latter variety of marrow may be produced experimentally by long-continued injections of hemolytic agents,[45] while the latter marrow can be produced only by toxins which apparently have no hemolytic effect, for example, benzol.[20]

Similarly, if secondary anemia as seen in severe infections, in malaria, in syphilis, in drug poisoning, etc., is due to excessive hemolysis of the red cells, the variations between it and aplastic anemia are most pronounced and the same arguments hold good as with the primary hemolytic anemias. If, however, secondary anemia is conceded to occur in which the toxicosis is one affecting the bone-marrow primarily and chiefly, then a very definite and close relationship between it and aplastic anemia can be drawn.

That the marrow changes in aplastic anemia are primary, I believe for the following reasons: The resistance of the red cells is not that of a hemolytic or pernicious anemia; the blood-picture, the chief characteristic of which is the total lack of evidence of any regeneration of red cells or formation of leukocytes, and the clinical courses are entirely dissimilar; while the marrow examination and other postmortem changes are very different from that of a hemolytic anemia, whether produced experimentally or seen at the necropsy of a patient dead of hemolytic or pernicious anemia. The main reason for the belief that aplastic anemia cannot exist as a primary disturbance of the bone-marrow is the fact that it has never been produced experimentally. Now, however, that Selling's[47] work on benzol poisoning has appeared, this fact cannot be brought as an argument against the existence of this disorder as a primary disturbance of the marrow.

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[46] Stone, W. J.: So-Called Aplastic Anemia and Its Relation to Progressive Pernicious Anemia, Ohio State Med. Jour., 1907, iii, 243.

Selling noted in these cases of benzol poisoning the typical picture of aplastic anemia which induced him to try experimentally to produce aplastic anemia with benzol and to study the effect of benzol poisoning.[20] In this work he shows that benzol is a powerful leukotoxin "but the circulating erythrocytes are injured relatively little." The degree of anemia produced experimentally is no more than could be accounted for by natural cell-death.

When the poison is given in repeated doses, the hemopoietic organs are rendered almost aplastic, so that in the long bones the final stages of the poisoning are accompanied by an almost complete aplasia of the marrow. As with arsenic and other destructive agents, the toxins act as a stimulant to the marrow, and here and there may be found small areas of hyperplastic marrow. I consider this an extremely important observation, because one of the important arguments against the entity of aplastic anemia is that in some of the post mortems in these cases, areas of active marrow are found[38] and hence aplastic marrow is considered to differ in degree only from that of the usual hemolytic anemia.

Gruner[25] considers the term "aplastic" as a misnomer because there must be some regeneration. This contention, that to have a true aplastic anemia there can be no regeneration of the red cells, is questionable. What toxin is there that will so act that an entire organ is at once rendered degenerative? Some parts of the organ must be more resistant than others, nor can the toxin spend itself equally on all parts.

If it has been shown experimentally that certain toxins can primarily affect the marrow (and I believe it has been so shown) without any evidence of blood destruction, there seems to be no necessity of speculating on various hypotheses which have not been experimentally demonstrated. Clinically, in a certain number of cases, I believe the myelotoxin has been demonstrated *intra vitam*. By that I mean that the cases of aplastic anemia that have been reported with a definite known cause,[13, 27, 3, 10, 21, 47, 48, 5] are due to toxins which spend themselves on the marrow alone. In several of these cases[27, 3] the toxin, of bacterial origin, may have been a specific poison to the marrow, just as benzol is. In the other cases, noxious agents which normally act in a certain way may have either become perverted so that, contrary to their usual action, they had a selective action on the marrow or else they acted on a marrow which was particularly susceptible or inherently weak, so that degenerative changes resulted before the usual manifestations of the toxin had time to express themselves.

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[47] Selling, L.: A Preliminary Report of Some Cases of Purpura Hemorrhagica Due to Benzol Poisoning, Johns Hopkins Hosp. Bull., 1910, xxi. 31.

SUMMARY

If we accept Pappenheim's[49] idea, a most rational and convincing theory, that all anemias are secondary, there can be no question but that aplastic anemia is a secondary anemia; an anemia, however, that is secondary to primary marrow-changes alone, and has no relation to the ordinary types of phanerogenetic anemias or to the so-called primary pernicious anemias, which are the result of primary hemolysis. Representing a type of hemopoietic degeneration, as such it should be considered as a nosologic entity and should not be considered merely a type of anemia secondary to some known or unknown hemolytic process. Aplastic anemia is an anemia secondary to marrow changes which in turn are dependent on some known or unknown cause.

1911 Pine Street.

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48. Lipowski: Beitrage zur Pathologie des Blutes, Deutsch. med. Wchnschr., 1900, xxvi, 340.

49. Pappenheim, A.: Foot note in Folia haematol., 1907, iv, supp. Bd., p. 320.

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INTRODUCTION TO THE SECOND PROGRESS REPORT OF THE THOMPSON-McFADDEN PELLAGRA COMMISSION *

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The investigations of the Thompson-McFadden Pellagra Commission have been directed especially toward the study of the etiological factors in pellagra. We conceive this problem to include not only a study of the nature of the disease, the recognition and identification of the essential infecting or intoxicating agent, or the recognition of the dietary elements, the deficiency of which may give rise to pellagra, but also necessarily a study of the disease in its endemic centers, a study of the people who live there, their habits of life, their environment and occupations and their food, and, especially, a careful scrutiny of those respects in which the non-pellagrins of such a population differ from the pellagrins living in the same regions.

During the course of this work we have undertaken to record every known case of pellagra existing within a limited geographical district,[1] namely, Spartanburg County, S. C., to examine personally each suspected case, and to record a detailed history of each pellagrin in whom a reliable diagnosis could be made. The records actually made in respect to each case are quite elaborate and include a complete history of the individual from birth, with particular attention to foods, occupations, residences, relationships and diseases, as well as a careful description of the present environment and life of the individual. By the aid of maps of Spartanburg County, and larger more detailed maps

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* From the Division of Tropical Medicine, Department of the Laboratories, New York Post-Graduate Medical School and Hospital.

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[1] The general plan of the epidemiological studies in 1912 has beeen outlined in detail in Section II of the First Progress Report. See Siler and Garrison: Am. Jour. Med. Sc., 1913, cxlvi, 42.

of the more thickly populated districts, each known case of pellagra has been located, and the houses known to have been occupied at any time by a pellagrin have been designated.

In intimate collaboration with this commission, the Bureau of Entomology of the United States Department of Agriculture has undertaken a comprehensive study of the insects which occur in the region studied, with special attention to their possible relationship to pellagra. This work included a general entomological survey of the territory, an examination of all insects found there in association with man, and a particular study of those species the biology of which suggested a possible relation to the spread of pellagra.

Detailed clinical and pathological studies were carried out on selected patients. Fourteen of these patients were transferred to the Post-Graduate Hospital in New York and treated there during the continuance of the active manifestations of the disease. During their residence in the hospital a careful study of their metabolism in general and, in particular, of the utilization of foods, was carried out. For hematological studies, blood was obtained from these patients and also from pellagrins at their homes in the South. The hospital cases also furnished an important part of the material for study of intestinal micro-organisms and for experimental inoculation of animals, although other cases have also been utilized in this work. Finally, these cases were the objects of a clinical study, and their hospital records present information concerning the course of the attack of pellagra when it is uninfluenced by attempts at specific medication.

Certain progress reports on the investigations of the commission in 1912 were presented at the National Pellagra Conference at Columbia, S. C., in October of that year, and these reports have been published in succeeding numbers of the *American Journal of the Medical Sciences*[2] from March to August, 1913. A summary of these reports was presented at the Pellagra Meeting in Spartanburg, S. C., Sept. 5, 1913 and appeared in the *Journal of the American Medical Association*,[3] Jan. 3, 1914. These papers have since been brought together and issued as the First Progress Report of the Thompson-McFadden Pellagra Commission.

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[2] Hillman, O. S.: Some Hematological Findings in Pellagra, Am. Jour. Med. Sc., 1913, cxlv, 507. Myers, V. C., and Fine, M. S.: Metabolism in Pellagra, ibid., 1913, cxlv, 705. MacNeal, W. J.: Observations on the Intestinal Bacteria in Pellagra, ibid., 1913, cxlv, 801. Siler, J. F., and Garrison, P. E.: An Intensive Study of the Epidemiology of Pellagra, Report of Progress, ibid., 1913, cxlvi, 42, 238. Jennings, A. H., and King, W. V.: An intensive Study of Insects as a Possible Etiologic Factor in Pellagra, ibid., 1913, clxvi, 411.

[3] Siler, J. F., Garrison, P. E., and MacNeal, W. J.: Pellagra: A Summary of the First Progress Report of the Thompson-McFadden Pellagra Commission, Jour. Am. Med. Assn., 1914, lxii, 8.

A considerable part of the work done in 1912 has not been included in these published reports for various reasons, but chiefly because of incompleteness of the work. This applies especially to the attempts to produce pellagra in animals by experimental inoculation, the clinical observations on the pellagrous patients treated in the hospital and the pathological study of necropsy material.

The work done in 1913 has been directly continuous with that of 1912, and supplements and extends the observations of that year. Our main efforts have been devoted to the epidemiologic studies, chiefly because these studies were expected to indicate the fruitful fields for other experimental investigations. The method of epidemiologic study has been continued along the same lines as before, and has been further developed in two special directions; first, we have endeavored to obtain more complete information concerning every person living in certain selected industrial and rural communities in which pellagra is prevalent; and second, we have extended our field of observation to cover other selected communities resembling these in many respects, but showing marked differences in certain particulars, as, for example, in the prevalence of pellagra; the presence or absence of certain insects; the possession of a topography which would necessitate a difference in insect fauna, in the density of population, or in the method of disposal of human wastes.

Inasmuch as the work of 1912 had shown an especially high prevalence of pellagra in the cotton-mill villages, several of these were selected for intensive detailed study, and one rural district in which many cases of pellagra had occurred was studied in a similar way. The communities selected for observation because they exhibited special features could not be examined in such detail, as in many instances they were situated at a distance from our working base. For this study we selected certain mill villages situated in Oconee, Chester and Union counties in South Carolina; a rural district in Georgetown County, on the coast of South Carolina; a large island off the coast near Charleston, S. C., and a portion of the arid region of Texas. At the courteous invitation of Dr. L. W. Sambon, we were enabled to extend these observations to the British West Indies.

The metabolism studies of 1912 had yielded definite negative information and seemed to be sufficiently complete and were therefore discontinued. The study of the blood has been continued and a further report will be forthcoming. Further observations on the intestinal micro-organisms have been made and the attempts to produce pellagra in animals have been zealously pushed. The clinical observations have been continued in cases of pellagra treated in a hospital in Spartanburg, S. C.

The cooperation of the Bureau of Entomology of the United States Department of Agriculture has continued during the past year. The work undertaken by this bureau has included a particularly careful study of insects in the endemic foci of pellagra and carefully controlled attempts at the experimental transmission of pellagra by means of certain of these insects.

We desire to make special acknowledgment to Dr. L. O. Howard, chief of the Bureau of Entomology, and to the field workers of the bureau, Mr. A. H. Jennings, Mr. W. V. King and Mr. A. W. J. Pomeroy, for this cooperation.

We are indebted to the American Genetic Association for the cooperation of its representative, Dr. Elizabeth Muncey, who has made a study of heredity in pellagra; to the Board of Administration of the state of Illinois for the collaboration of Dr. H. Douglas Singer, who spent several weeks with us studying the early nervous and mental manifestations of pellagra; to Dr. J. W. Babcock of Columbia, S. C., for generous assistance and valuable material; to Dr. E. M. Green, clinical director of the Georgia State Sanitarium, Milledgeville, Ga., and to members of his staff for similar cooperation; to Dr. J. A. Hayne, secretary of the South Carolina State Board of Health, for much valuable information and assistance; to Dr. L. W. Sambon of London, and to the government officials of Jamaica, Panama, Barbados and Trinidad, for courteous assistance and extensive records relating to pellagra.

Our field laboratory has been visited during 1913 by a number of noted scientists and students of pellagra; among them Dr. J. W. Babcock of the South Carolina State Hospital, Columbia, S. C.; Surg.-Gen. Rupert Blue of the United States Public Health Service, Washington, D. C.; P. A. Surg. R. M. Grimm of the United States Public Health Service; Dr. W. D. Hunter of the Bureau of Entomology, United States Department of Agriculture; Mr. M. E. MacGregor of Cambridge University, Cambridge, England; Mr. Paul Marchal of the French Department of Agriculture, Paris; Mr. C. W. Mason of Cambridge University, Cambridge, England; Dr. George N. Miller of the New York Post-Graduate Medical School and Hospital, New York; Drs. G. M. Niles, S. R. Roberts and G. C. Mizell of Atlanta, Ga.; Dr. L. W. Sambon of the London School of Tropical Medicine, London; and Dr. E. J. Wood of Wilmington, N. C. We are grateful to these men for their encouragement and counsel.

We desire to make special acknowledgment to Dr. Paul A. Schule, whose addition to the staff of the commission has contributed much to the success of the year's work.

It is our purpose to present, as the Second Progress Report of this commission, a series of papers by the various investigators, dealing with several phases of the work of the year 1913.

Twentieth Street and Second Avenue.

A STATISTICAL STUDY OF THE RELATION OF PELLAGRA TO USE OF CERTAIN FOODS AND TO LOCATION OF DOMICILE IN SIX SELECTED INDUSTRIAL COMMUNITIES *

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AND
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Our most intensive epidemiologic studies have been carried out in six cotton-mill villages in Spartanburg County, S. C., selected as fairly representative of the many mill villages in that part of the country. In each of these villages the family of each operative was visited at his home and a complete record was taken on a uniform printed card, of the location of the house, the sex, age, relationship, occupation, period of present residence and association with pellagra, of each inhabitant. On the reverse side of the same card was recorded the frequency of use of certain important elements of the diet and the condition of the house in respect to screening and general condition.

Whenever a case of pellagra was found, the usual complete history of the individual was taken, and this included a record of the previous residences of the individual, the time of the first attack and a very complete record of his diet. These records make possible statistical studies of the distribution of pellagra in these populations to ascertain its correlation with various other things, as, for example, age, sex, occupation, use of particular foods, location of the domicile or sanitary condition of the home. They seem to us to be of peculiar value for the study of the influence of foods on pellagra and for an inquiry as to the possible bearing which location of domicile may have in the causation of this disease. In the present paper we purpose to present, first, a study of the relation of pellagra to certain foods, and second, a study of the relation between the domicile of existing cases of pellagra and the domicile of the new cases which originated in

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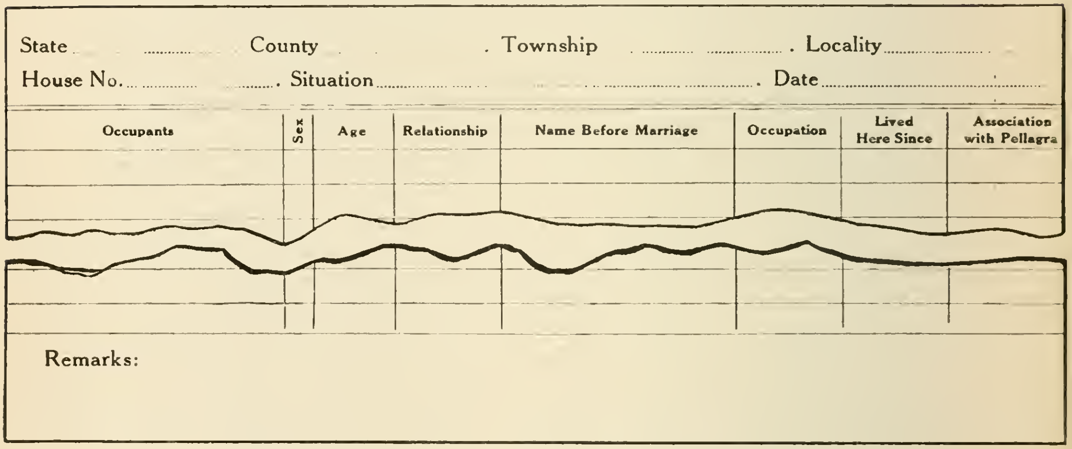
these villages. The two studies have been carried out by much the same method, and because of their interrelationships and the sharp contrast in their results, it has been thought best to present them in a single contribution.

THE RELATION OF PELLAGRA TO THE USE OF PARTICULAR FOODS

In view of the apparent importance of nutrition as an etiological factor in pellagra, and because of the confident belief of many authorities that the disease is due to deficiency in diet, either general or special, or that it is a specific food intoxication, we have undertaken a careful investigation of the dietary habits, not only of pellagrins and their families, but also of all the remaining population of the same class living under the same conditions, in certain selected industrial communities. Approximately five thousand persons have been included in these studies. The facts were ascertained for each family by personal interview with a member of the family, usually the housewife. We recorded the diet served in the family as a whole, and each member of the household is considered as belonging to the dietary group of his family. For each pellagrin, the individual diet was recorded in detail, but for the comparative study which we present in this section, each of these persons is considered as belonging to the dietary group of his family, not only because this makes them strictly comparable to the other individuals, but also because the individual peculiarities in the diet of pellagrins, particularly the omission of corn from the diet, may often be regarded as secondary to the development of the disease.



State .... County .... Township .... Locality ....

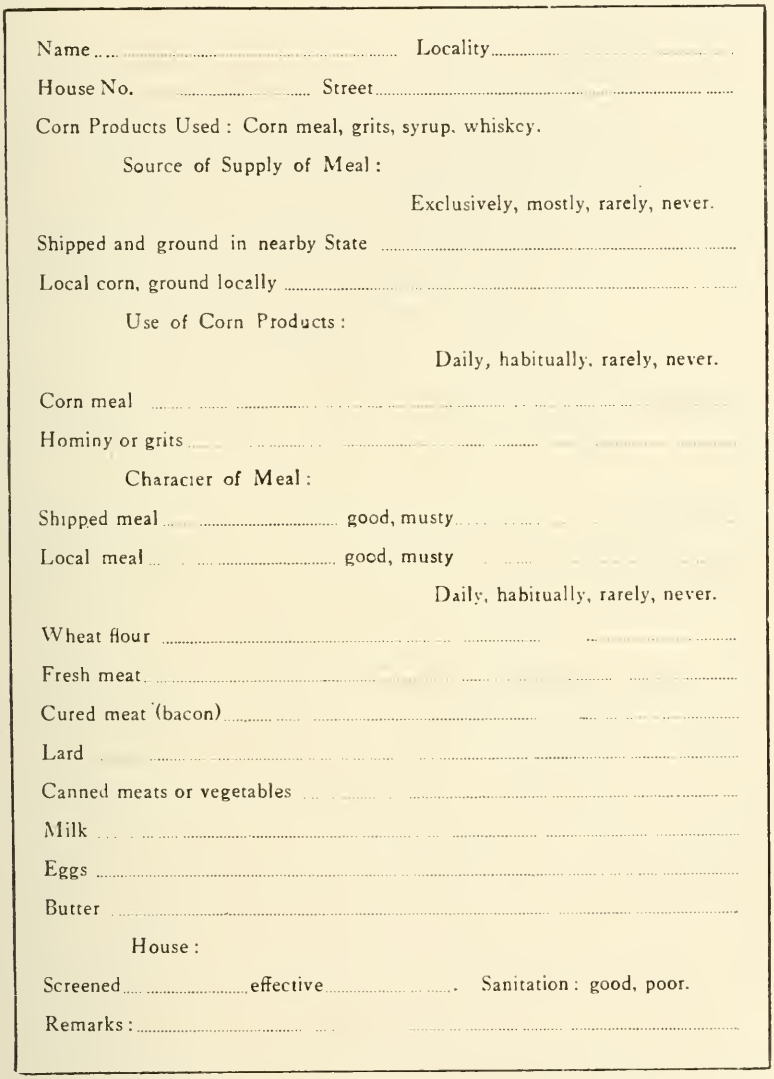
House No. .... Situation .... Date ....

Occupants	Sex	Age	Relationship	Name Before Marriage	Occupation	Lived Here Since	Association with Pellagra

Remarks:

Fig. 1.—Census card used in the house-to-house canvas.

In obtaining the data a uniform printed card was used and the record was made concerning the frequency of the use of shipped corn-meal, locally grown corn-meal, and the quality of the meal used; concerning the use of grits, corn-sirup, corn whisky, wheat flour, fresh meats, cured meats, canned meats, vegetables and fruits (tinned foods), and concerning leaf-lard, compound lard, vegetable oils, milk, butter and eggs. In respect to the frequency of use, we recognized seven classes: first, daily use which is self-explanatory; second, habitual use,



Name .... Locality ....

House No. .... Street ....

Corn Products Used: Corn meal, grits, syrup, whiskey.

Source of Supply of Meal:

Exclusively, mostly, rarely, never.

Shipped and ground in nearby State ....

Local corn, ground locally ....

Use of Corn Products:

Daily, habitually, rarely, never.

Corn meal ....

Hominy or grits ....

Character of Meal:

Shipped meal .... good, musty ....

Local meal .... good, musty ....

Daily, habitually, rarely, never.

Wheat flour ....

Fresh meat ....

Cured meat (bacon) ....

Lard ....

Canned meats or vegetables ....

Milk ....

Eggs ....

Butter ....

House:

Screened .... effective .... Sanitation: good, poor.

Remarks: ....

Fig. 2.—Reverse side of the census card.

meaning as often as twice a week on the average; third, part time daily, which means daily use during certain seasons of the year; fourth, part time habitually, or habitual use during certain seasons; fifth, rarely, which means use less frequently than twice a week; sixth, part time rarely; and seventh, never.

DIETARY ELEMENTS DISMISSED FROM CONSIDERATION

Certain of these articles were not very commonly used and there is evidently no important reason for giving them detailed consideration. This applies particularly to corn-grits and corn-sirup. Corn whisky was not used by many, possibly in part because of legal restriction of the liquor traffic in the territory studied. For the most part, it was used only by the adult males, a class of the population in which pellagra was not very prevalent. It has not, therefore, seemed worth while to assemble and analyze the data in regard to it. Wheat flour was used daily by every family in the population studied. No distinction between pellagrins and non-pellagrins could be ascertained in respect to this dietary element. The recorded data in respect to lard are difficult to analyze because so many different brands were used and we have not, as yet, obtained reliable information concerning their composition. The remaining foods concerning which information has been gathered, namely, shipped corn-meal, local corn-meal, fresh meat, canned (tinned) foods, milk and eggs will be considered.

METHOD OF PRESENTATION

The general method of analyzing these data consists in dividing the population into several groups, distinguished from each other by the frequency with which the particular food was used, and then comparing the relative number of cases of pellagra in the different groups. Four distinct possibilities present themselves. We may consider as a unit each family present in the village at the time of our census, and compare the families in which pellagra existed with the total families present, or we may consider each individual in the same population as a unit and compare the total existing pellagrins with the total population. These two analyses might be expected to agree with each other fairly well, and should indicate the possible relation of diet to the presence of pellagra, both new and recurrent cases being considered together. Third, we may consider as a unit each family of which we have record, which lived in the particular village in 1912 or 1913 and compare the families in which *new cases* of pellagra appeared, with the total families, and fourth, we may examine the same population in a similar way, considering each individual person as a unit. The last two analyses might be expected to agree with each other and should reveal the relation of these dietary constituents to new cases of pellagra. The latter would probably be a more accurate indication of the importance of the particular foods as predisposing factors, or as vehicles of the directly active etiological agents. Because of the manifest importance of this dietary phase of the subject of pellagra, we have undertaken to study our data in all four of the ways mentioned.

SHIPPED CORN-MEAL

The records in regard to the use of shipped corn include 853 families, present in the six villages in the year 1913, in 137 of which one or more pellagrins existed at the time. The distribution of these 853 families in respect to the frequency of use of shipped corn-meal is indicated in Table 1.

TABLE 1.—Distribution of Total Families According to Frequency of Use of Shipped Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	47	14	8	11	5	2	15	102
W	44	18	4	16	2	0	11	95
P	116	51	22	12	2	1	18	222
Sa	38	15	10	10	6	3	19	101
A	33	22	14	6	1	2	4	82
Sp	130	40	27	14	8	1	31	251
Total..	408	160	85	69	24	9	98	853

It will be noted that this foodstuff was a staple article of diet, used daily by nearly half the families and never used by only about 11 per cent. of them. There was evidently not much difference between the various villages in the use of this food. At the larger mills, P and Sp, more than half the families used it daily, while in one village, Sa, only 38 of 101 families did so. The other villages are intermediate in this respect.

The distribution of the 137 families in which pellagra was present, in respect to frequency of use of shipped corn-meal, is indicated in Table 2.

By grouping together those families using shipped corn-meal habitually, part time daily and part time habitually under the heading, *Habitually,* and the groups using it rarely, part time rarely and never, together under the heading *Rarely,* Tables 3 and 4 are obtained.

TABLE 2.—FAMILIES CONTAINING ONE OR MORE CASES OF PELLAGRA DISTRIBUTED ACCORDING TO FREQUENCY OF USE OF SHIPPED CORN-MEAL

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	5	3	1	2	0	0	2	13
W	10	6	1	1	0	0	1	19
P	10	9	5	2	0	0	2	28
Sa	11	4	2	3	1	2	6	29
A	7	5	4	0	0	0	1	17
Sp	13	6	2	5	1	1	3	31
Total..	56	33	15	13	2	3	15	137

TABLE 3.—SUMMARIZED DISTRIBUTION OF FAMILIES ACCORDING TO FREQUENCY OF USE OF SHIPPED CORN-MEAL

Village	Total Families			Pellagrin Families		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	47	30	25	5	5	3
W	44	36	15	10	7	2
P	116	65	41	10	11	7
Sa	38	31	32	11	8	10
A	33	29	20	7	5	5
Sp	130	62	59	13	12	6
Total	408	253	192	56	48	33

TABLE 4.—PELLAGRIN FAMILIES IN TOTAL FAMILIES IN EACH OF THE SUMMARIZED GROUPS, PER CENT.

Village	Daily	*Habitually*	*Rarely*	Total
I	10.6	16.7	12.0	12.7
W	22.7	19.4	13.3	20.0
P	8.6	16.9	17.1	12.6
Sa	28.9	25.8	31.3	28.7
A	21.2	17.2	25.0	20.7
Sp	10.0	19.4	10.2	12.4
Total.........	13.7	19.0	17.2	16.1

The large villages, Sp and P, show relatively fewer families with cases of pellagra, while in the village Sa, in which shipped corn-meal was less frequently used, nearly 29 per cent. of the families contained one or more cases. In three of the villages, P, Sa and A, the families using shipped meal rarely or never were most frequently afflicted with pellagra. In only one village, W, is the percentage of pellagrin families highest in the group using this food daily, and for the total families of the six villages this group shows relatively the least pellagra. The figures are rather irregular and inconsistent, but on the whole they seem to us to indicate that families containing one or more pellagrins tend in some instances to restrict their use of shipped corn-meal. The tables do not reveal any positive correlation between the use of this food and the occurrence of pellagra.

The 853 families just considered included 5,151 persons, among whom 196 were pellagrins. The distribution of these 5,151 persons in respect to the frequency of use of shipped corn-meal is shown in Table 5.

TABLE 5.—Distribution of Total Individuals According to Frequency of Use of Shipped Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	286	75	38	71	40	13	65	588
W	275	92	20	90	14	0	74	565
P	737	331	121	74	16	5	82	1,366
Sa	244	101	49	63	35	20	123	635
A	171	138	102	81	3	7	23	525
Sp	800	225	151	88	44	4	160	1,472
Total..	2,513	962	481	467	152	49	527	5,151

On the whole, approximately half these persons were members of families in which shipped corn-meal was used daily. In the large villages, P and Sp, more than half the persons were members of such families, while in the village A, less than one-third of the population took this food daily, and in Sa, considerably less than half. The distribution of the 196 pellagrins in this population in respect to the frequency of use of shipped corn-meal is given in Table 6.

A consideration of the pellagra morbidity in each village for each of the seven degrees of frequency in the use of shipped corn-meal is omitted because of the small size of certain of the groups. By assembling the figures into groups representing three degrees of frequency, namely, daily, *habitually* and *rarely* (including never) it is possible to obtain groups of some size (Tables 7 and 8).

TABLE 6.—Distribution of Pellagrins According to Frequency of Use of Shipped Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	8	3	1	3	0	0	4	19
W	11	7	3	2	0	0	1	24
P	10	11	8	4	0	0	4	37
Sa	16	4	2	3	2	3	13	43
A	12	7	7	0	0	0	1	27
Sp	19	9	5	6	1	1	5	46
Total..	76	41	26	18	3	4	28	196

TABLE 7.—Summarized Distribution According to Frequency of Use of Shipped Corn-Meal

Village	Total Population			Individual Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	286	186	116	8	6	5
W	275	196	94	11	9	4
P	737	421	208	10	15	12
Sa	244	199	192	16	9	18
A	171	222	132	12	7	8
Sp	800	357	315	19	16	11
Total	2,513	1,581	1,057	76	62	58

TABLE 8.—Pellagra Morbidity in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	2.80	3.22	4.31	3.23
W	4.00	4.59	4.26	4.25
P	1.36	3.56	5.77	2.71
Sa	6.56	4.52	9.37	6.77
A	7.02	3.15	6.06	5.14
Sp	2.38	4.48	3.49	3.13
Total.........	3.02	3.92	5.49	3.81

The comparative study of pellagra morbidity in these groups for each of the six villages shows no definite consistent tendency for it to vary with the frequency of use of shipped corn-meal. In three villages, I, P and Sp, and in the total population of the six villages considered together, the pellagra morbidity varies inversely with the frequency of ingestion of this food, that is, those who eat it every day have the least pellagra, and those persons taking it rarely show the greatest morbidity. In the other three villages the relationship is irregular. The total pellagra morbidity in the larger villages, P and Sp, in which shipped corn-meal was most extensively used, is the lowest, namely, 2.71 and 3.13 per cent., respectively, while the greatest relative morbidity is shown by the villages Sa and A, in which shipped corn-meal was least used. This consideration of individuals leads, therefore, to the same general conclusion as did the consideration of the families, namely, that there is slightly more pellagra in those who use shipped corn less frequently. As has been suggested, this may be due to a tendency of some pellagrins to restrict their ingestion of this food. At any rate it is clearly evident that this examination of our recorded data has failed to reveal any close connection between the use of shipped corn-meal and the existence of pellagra in the population of these six mill villages.

In order to ascertain more exactly the probable importance of shipped corn-meal in the causation of pellagra, we have undertaken a study of cases of the disease which originated in these six mill villages during the period of our observations (1912 and 1913). In this study we have included all families residing in the particular village at the time of our census in 1913, and in addition all other families on our records in which cases of pellagra originated in the respective village in 1912 or 1913. The population considered is therefore not exactly the same as that utilized above, but it is very largely identical with it. The records include 865 families, and in 85 of these families one or more cases of pellagra developed in 1912 or 1913.

The distribution of these 865 families according to the frequency of use of shipped corn-meal is indicated in Table 9.

Table 9 is very similar to Table 1, but includes a few more families which had moved away from the respective village after one or more of their members had contracted pellagra there. The distribution of the 85 families in which cases of pellagra originated in 1912 or 1913 in these villages is given in Table 10.

Assembling the families into three groups in respect to frequency of use of shipped corn-meal, the figures in Table 11 are obtained.

There is evidently no consistent relationship between the frequency of the use of shipped corn-meal and the occurrence of new cases of

TABLE 9.—Distribution of Total Families According to Frequency of Use of Shipped Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	49	15	8	11	6	2	15	106
W	45	18	4	16	2	1	12	98
P	116	51	22	12	2	1	18	222
Sa	40	15	10	10	6	3	19	103
A	33	22	14	6	1	3	4	83
Sp	131	40	27	14	8	1	32	253
Total..	414	161	85	69	25	11	100	865

TABLE 10.—Distribution of Families in Which New Cases of Pellagra Occurred in 1912 or 1913, According to Frequency of Use of Shipped Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	7	3	1	2	1	0	2	16
W	6	1	0	1	0	1	2	11
P	8	4	5	1	0	0	1	19
Sa	6	2	0	0	1	2	1	12
A	3	4	2	0	0	1	0	10
Sp	8	3	1	2	0	0	3	17
Total..	38	17	9	6	2	4	9	85

TABLE 11.—Summarized Distribution of Families According to Frequency of Use of Shipped Corn-Meal

Village	Total Families			Families with Incident Pellagra		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	49	32	25	7	6	3
W	45	36	17	6	2	3
P	116	65	41	8	5	6
Sa	40	31	32	6	3	3
A	33	29	21	3	4	3
Sp	131	62	60	8	5	4
Total	414	255	196	38	25	22

pellagra to be seen in these tables. The figures for the six villages combined suggest rather that new cases were slightly more frequent in families using this food product rarely or never. Here also, the lowest incidence of pellagra is in the large villages, P and Sp, the only ones in which shipped corn-meal was used daily by more than half the families.

TABLE 12.—Families with Incident Pellagra in Total Families in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	14.3	18.8	12.0	15.1
W	13.3	5.6	17.6	11.2
P	6.9	7.7	14.6	8.6
Sa	15.0	9.7	9.4	11.7
A	9.1	13.8	14.3	12.0
Sp	6.1	8.1	6.7	6.7
Total.........	9.2	9.8	11.2	9.8

TABLE 13.—Distribution of Individuals According to Frequency of Use of Shipped Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	300	89	38	71	44	13	65	620
W	272	86	20	89	14	6	77	564
P	736	324	119	72	16	5	79	1,351
Sa	248	99	47	60	34	20	112	620
A	165	135	99	81	3	12	23	518
Sp	794	219	149	84	43	3	165	1,457
Total..	2,515	952	472	457	154	59	521	5,130

The 865 families just considered included 5,130 persons who did not have pellagra at the beginning of the year 1912, so far as we have been able to ascertain. Persons known to have contracted pellagra previous to 1912 have been excluded from this number. Of these 5,130 persons, 110 contracted pellagra during the years 1912 and 1913. The distribution of the 5,130 individuals of this population in respect to the frequency of the use of shipped corn in their families is shown in Table 13.

Table 13 resembles Table 5, but the population considered is not quite the same. Old cases of pellagra existing previous to 1912 are excluded from this table and some other persons have been included who, although not residing in these villages at the time of our census in 1913, nevertheless did reside there in 1912 or 1913 when some member of their family contracted pellagra. The 110 persons who contracted pellagra in these villages in 1912 and 1913 were distributed according to frequency of use of shipped corn by their families as follows:

TABLE 14.—Distribution of Incident Pellagrins According to Frequency of Shipped Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	10	3	1	3	1	0	4	22
W	6	1	0	1	0	3	2	13
P	9	4	6	2	0	0	1	22
Sa	6	2	0	0	1	3	2	14
A	6	4	4	0	0	1	0	15
Sp	11	3	3	2	0	0	5	24
Total..	48	17	14	8	2	7	14	110

By assembling these figures into the groups representing the three degrees of frequency of use of shipped corn-meal, Tables 15 and 16 are obtained.

It is clearly evident, from these tables, that the occurrence of new cases of pellagra in these mill villages shows no definite consistent tendency to vary with the frequency of use of shipped corn-meal. On the contrary, in every one of these six villages the incidence of the disease actually observed was highest in that portion of the population which rarely or never used this foodstuff. In the total population of the six villages the occurrence of new cases of pellagra was almost twice as frequent in those using shipped meal rarely as in the groups using it daily or habitually. These results lend no support to the conception that pellagra is caused by the ingestion of shipped corn-meal in these particular mill villages and they seem to us to render very insecure the theory that imported or shipped corn may anywhere be regarded as the essential cause of pellagra.

LOCAL CORN-MEAL

The recorded data in regard to the use of local or home-grown corn-meal relate to 866 families present at the time of our census, of which 134 contained one or more cases of pellagra at that time. The distribution of these 866 families in respect to the frequency of use of local corn-meal is given in Table 17.

TABLE 15.—SUMMARIZED DISTRIBUTION ACCORDING TO FREQUENCY OF USE OF SHIPPED CORN-MEAL

Village	Total Population			Incident Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	300	204	116	10	7	5
W	272	189	103	6	2	5
P	736	412	203	9	6	7
Sa	248	193	179	6	3	5
A	165	219	134	6	4	5
Sp	794	346	317	11	5	8
Total	2,515	1,563	1,052	48	27	35

TABLE 16.—INCIDENCE OF PELLAGRA IN EACH OF THE SUMMARIZED GROUPS, PER CENT.

Village	Daily	*Habitually*	*Rarely*	Total
I	3.33	3.43	4.31	3.55
W	2.21	1.06	4.85	2.30
P	1.22	1.46	3.45	1.63
Sa	2.42	1.55	2.79	2.26
A	3.64	1.83	3.73	2.90
Sp	1.39	1.44	2.52	1.65
Total.........	1.91	1.73	3.33	2.14

TABLE 17.—DISTRIBUTION OF TOTAL FAMILIES ACCORDING TO FREQUENCY OF USE OF LOCAL CORN-MEAL

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	5	7	1	11	5	2	72	103
W	8	3	1	16	3	0	77	108
P	8	4	4	12	2	1	192	223
Sa	10	7	2	10	6	3	60	98
A	0	1	4	7	1	2	67	82
Sp	5	3	2	13	7	1	221	252
Total..	36	25	14	69	24	9	689	866

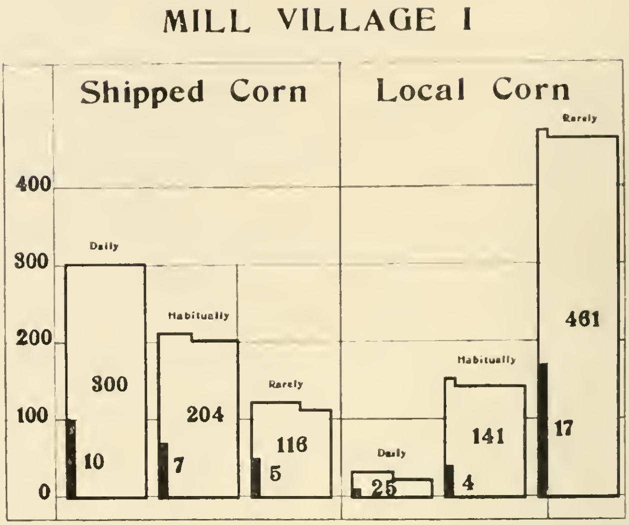


Fig. 3.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

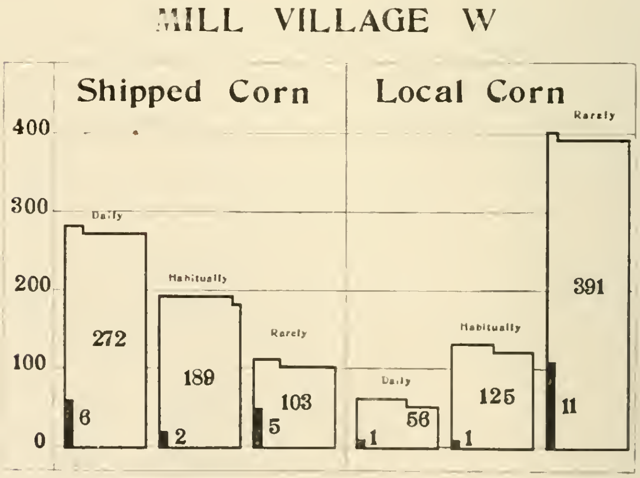


Fig. 4.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

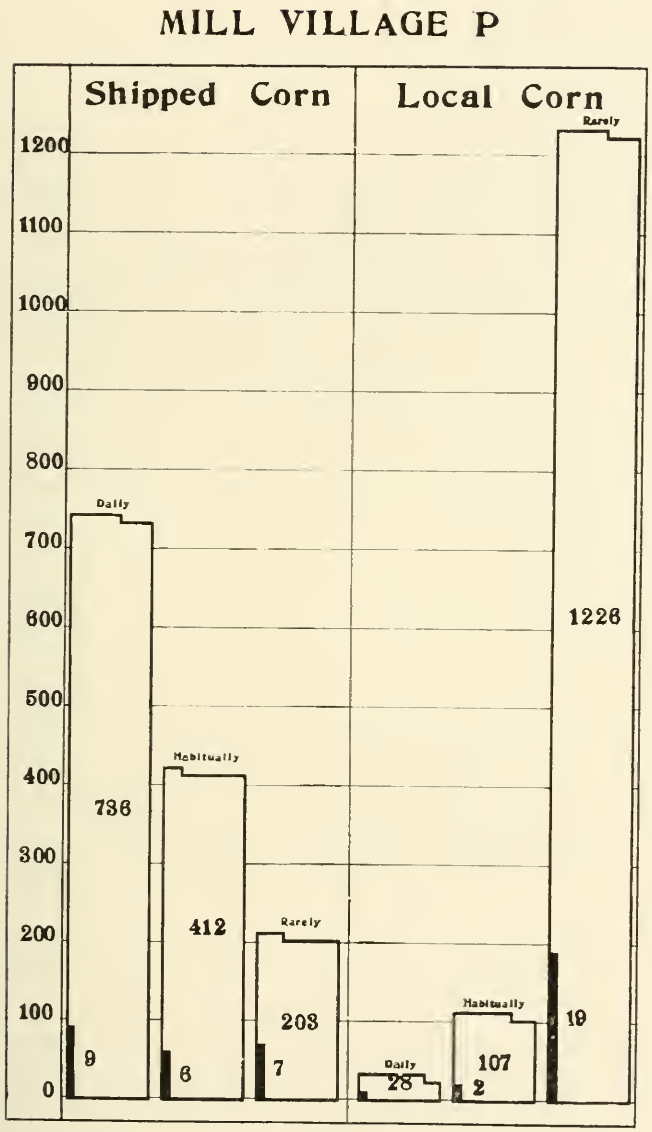


Fig. 5.—The area of each large white column indicates the number of non-pellagrin individauls using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

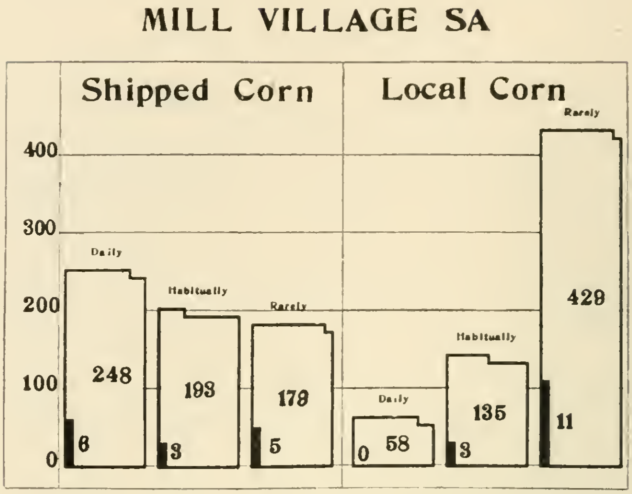


Fig. 6.—The area of each large white column indicates the number of nonpellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

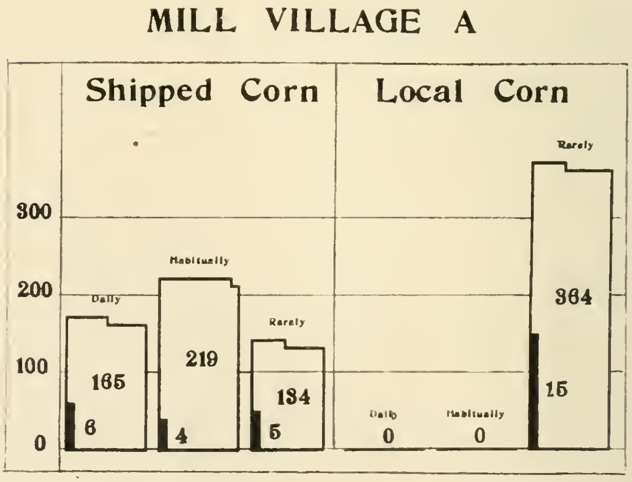


Fig. 7.—The area of each large white column indicates the number of nonpellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

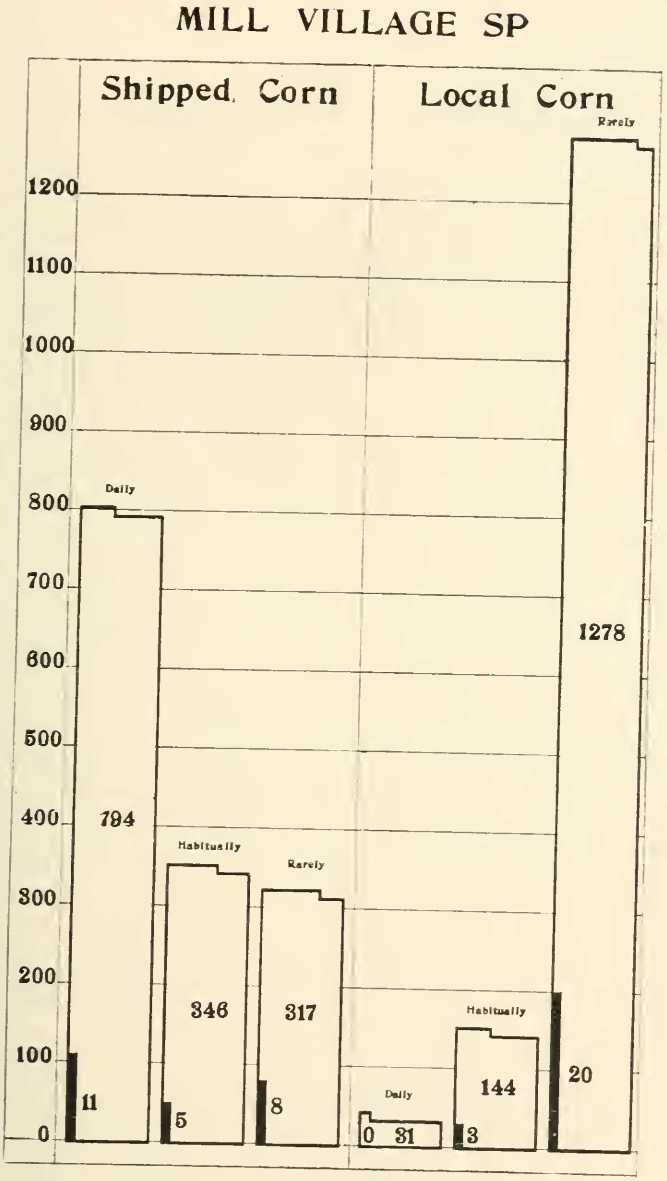


Fig. 8.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

It will be noted at once that local corn was not very extensively used. More than three-quarters of the total families never used it. It was most largely used in the village Sa, and used by relatively least number of families in the large villages of P and Sp. The distribution of the 134 families containing one or more cases of pellagra in respect to the frequency of use of local corn is shown in Table 18.

By assembling these figures into the usual three summarized groups in respect to frequency of use of the food, the figures shown in Table 19 are obtained.

It will be noted that the percentage of families having one or more cases of pellagra was highest in the village Sa, namely, 24.5 per cent., in which, as we have seen above, local corn-meal was most extensively used; and was lowest in the large villages, P and Sp, in which this foodstuff was used the least. This is undoubtedly due, in our opinion, to a tendency of some families living in pellagrous districts to restrict their use of shipped corn and substitute for it, in whole or in part, local corn-meal. The theories concerning causation of the disease which may be held by the local physicians or by the people themselves also seems to be reflected to some extent in the food data. In respect to frequency of use of local corn-meal in relation to the existence of pellagra, the data indicate no particular connection. The highest percentage of pellagrin families occurred in those using this food rarely or never, but the percentage in those who never used it, namely, 15.4, was practically the same as the general percentage for all families, namely, 15.5. In general those families using local corn-meal daily had pellagra less frequently, but the number of families in this group (namely, 36) is, after all, too small to be of much significance. It does show, however, that families using local corn-meal daily and avoiding shipped meal altogether, nevertheless did suffer from pellagra.

These 866 families were made up of 5,089 persons of whom 194 were pellagrins. The distribution of the 5,089 persons and of the 194 pellagrins in respect to the frequency of use of local corn-meal is shown in Tables 21, 22, 23 and 24.

It is evident that the frequency of use of local corn-meal has no definite consistent relation to the pellagra morbidity. In the whole population of the six villages, the 201 persons using it daily show a somewhat smaller proportionate number of cases than the larger group using it rarely or never. Yet, in two villages, I and P, the highest morbidity was among those using this food daily; in two others, Sa and Sp, the highest morbidity was among those using it habitually, and in the remaining two villages, W and A, those persons using local corn rarely or never had the most pellagra. This food

TABLE 18.—FAMILIES CONTAINING ONE OR MORE CASES OF PELLAGRA, DISTRIBUTED ACCORDING TO FREQUENCY OF USE OF LOCAL CORN-MEAL

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	1	0	1	2	0	0	10	14
W	1	0	0	1	0	0	18	20
P	1	1	0	2	0	0	24	28
Sa	1	4	0	3	1	2	13	24
A	0	0	2	0	0	0	15	17
Sp	0	0	0	4	0	1	26	31
Total..	4	5	3	12	1	3	106	134

TABLE 19.—SUMMARIZED DISTRIBUTION OF FAMILIES ACCORDING TO FREQUENCY OF USE OF LOCAL CORN-MEAL

Village	Total Families			Pellagrin Families		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	5	23	75	1	2	11
W	8	22	78	1	1	18
P	8	18	197	1	3	24
Sa	10	23	65	1	8	15
A	0	9	73	0	0	17
Sp	5	23	224	0	4	27
Total	36	118	712	4	18	112

TABLE 20.—PELLAGRIN FAMILIES IN TOTAL FAMILIES IN EACH OF THE SUMMARIZED GROUPS, PER CENT.

Village	Daily	*Habitually*	*Rarely*	Total
I	20.0	8.7	14.7	13.6
W	12.5	4.5	23.1	18.5
P	12.5	16.7	12.2	12.6
Sa	10.0	34.8	23.1	24.5
A	....	0.0	23.3	20.7
Sp	0.0	17.4	12.1	12.3
Total.........	11.1	15.3	15.7	15.5

TABLE 21.—DISTRIBUTION OF TOTAL INDIVIDUALS ACCORDING TO FREQUENCY OF USE OF LOCAL CORN-MEAL

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	25	26	8	71	40	13	413	596
W	56	22	4	90	14	0	389	575
P	30	20	21	74	16	5	1,210	1,376
Sa	59	47	8	63	35	20	404	636
A	0	11	18	45	3	7	352	436
Sp	31	20	12	84	37	4	1,282	1,470
Total..	201	146	71	427	145	49	4,050	5,089

TABLE 22.—DISTRIBUTION OF PELLAGRINS ACCORDING TO FREQUENCY OF USE OF LOCAL CORN-MEAL

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Dailv	Habitually	Rarely		
I	1	0	3	3	0	0	13	20
W	1	0	0	2	0	0	20	23
P	3	1	0	4	0	0	29	37
Sa	1	8	0	3	2	3	23	40
A	0	0	2	0	0	0	25	27
Sp	0	0	0	5	0	1	41	47
Total..	6	9	5	17	2	4	151	194

TABLE 23.—SUMMARIZED DISTRIBUTION ACCORDING TO FREQUENCY OF USE OF LOCAL CORN-MEAL

Village	Total Population			Individual Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	25	137	434	1	3	16
W	56	126	393	1	2	20
P	30	110	1,236	3	5	29
Sa	59	145	432	1	13	26
A	0	59	377	0	0	27
Sp	31	141	1,298	0	5	42
Total	201	718	4,170	6	28	160

was most commonly used in the village Sa, in which pellagra was very prevalent, and was used hardly at all in village A, in which pellagra morbidity was almost as high.

It remains now to study the population free from pellagra at the beginning of the year 1912 and the cases which developed in this population during 1912 and 1913, concerning which we have data in regard to the frequency of use of local corn-meal. The distribution of the total families (875) in this population and of the families in which new cases of pellagra occurred (84) are shown in Tables 25, 26, 27 and 28.

TABLE 24.—Pellagra Morbidity in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	4.00	2.19	3.69	3.36
W	1.79	1.59	5.09	4.00
P	10.00	4.55	2.35	2.69
Sa	1.69	8.97	6.02	6.29
A	....	0.00	7.16	6.19
Sp	0.00	3.55	3.24	3.20
Total.........	2.99	3.90	3.84	3.81

TABLE 25.—Distribution of Total Families According to Frequency of Use of Local Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	5	7	1	11	6	2	75	107
W	8	3	1	16	2	1	79	110
P	8	4	4	12	2	1	192	223
Sa	10	7	2	10	6	3	62	100
A	0	1	2	7	1	3	67	81
Sp	5	4	2	13	7	1	222	254
Total..	36	26	12	69	24	11	697	875

Approximately 80 per cent. of the families here considered did not use local corn-meal, so that the groups using it are composed of only a few families each. It is perfectly evident, however, that new cases of pellagra occurred in one or more families in every group without any definite consistent relation to the frequency of use of local corn-meal.

TABLE 26.—Distribution of Families in which New Cases of Pellagra Occurred in 1912 and 1913, According to Frequency of Use of Local Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	1	0	1	2	1	0	11	16
W	1	0	0	1	0	1	8	11
P	1	0	0	1	0	0	17	19
Sa	0	1	0	0	1	2	8	12
A	0	0	0	0	0	1	9	10
Sp	0	1	0	2	0	0	13	16
Total..	3	2	1	6	2	4	66	84

TABLE 27.—Summarized Distribution of Families According to Frequency of Use of Local Corn-Meal

Village	Total Families			Families with Incident Pellagra		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	5	24	78	1	3	12
W	8	21	81	1	1	9
P	8	18	197	1	1	17
Sa	10	23	67	0	2	10
A	0	9	72	0	0	10
Sp	5	24	225	0	3	13
Total	36	119	720	3	10	71

TABLE 28.—Families with Incident Pellagra in Total Families in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	20.0	12.5	15.4	15.0
W	12.5	4.8	11.1	10.0
P	12.5	5.6	8.6	8.5
Sa	0.0	8.7	14.9	12.0
A	....	0.0	13.9	12.3
Sp	0.0	12.5	5.8	6.3
Total.........	8.3	8.4	9.9	9.6

In the 875 families just considered, there were 4,999 persons who were not pellagrins, so far as we could ascertain, at the beginning of the year 1912. Of these 4,999 persons, 109 contracted the disease during 1912 and 1913. The distribution according to the use of local corn-meal in their families, of the 4,999 total individuals and of the 109 who became pellagrins in 1912 and 1913, is shown in Tables 29, 30, 31 and 32.

TABLE 29.—DISTRIBUTION OF INDIVIDUALS ACCORDING TO FREQUENCY OF USE OF LOCAL CORN-MEAL

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	25	26	8	71	44	13	440	627
W	56	22	4	89	14	6	381	572
P	28	19	21	72	16	5	1,200	1,361
Sa	58	41	8	60	34	20	401	622
A	0	0	0	0	0	12	352	364
Sp	31	26	12	81	37	3	1,263	1,453
Total..	198	134	53	373	145	59	4,037	4,999

TABLE 30.—DISTRIBUTION OF INCIDENT PELLAGRINS ACCORDING TO FREQUENCY OF USE OF LOCAL CORN-MEAL

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	1	0	3	3	1	0	14	22
W	1	0	0	1	0	3	8	13
P	1	0	0	2	0	0	19	22
Sa	0	2	0	0	1	3	8	14
A	0	0	0	0	0	1	14	15
Sp	0	1	0	2	0	0	20	23
Total..	3	3	3	8	2	7	83	109

There is a suggestion of an inverse relation between the use of local corn-meal and the occurrence of new cases of pellagra in this population as a whole, those using it daily showing an incidence of new cases of 1.52 per cent., whereas those using it rarely or never show an incidence of 2.24 per cent. In village A, in which local corn-meal scarcely entered into the diet, the highest incidence of new cases was

TABLE 31.—Summarized Distribution According to Frequency of Use of Local Corn-Meal

Village	Total Individuals			Incident Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	25	141	461	1	4	17
W	56	125	391	1	1	11
P	28	107	1,226	1	2	19
Sa	58	135	429	0	3	11
A	0	0	364	0	0	15
Sp	31	144	1,278	0	3	20
Total	198	652	4,149	3	13	93

TABLE 32.—Incidence of Pellagra in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	4.00	2.84	3.69	3.51
W	1.79	0.80	2.81	2.27
P	3.57	1.87	1.55	1.62
Sa	0.00	2.22	2.56	2.25
A	....	....	4.12	4.12
Sp	0.00	2.08	1.56	1.58
Total.........	1.52	1.99	2.24	2.18

observed. In two villages, however, namely, I and P, the incidence was highest in the group using local corn-meal daily. Probably several conflicting factors disturb the relationships. In general we believe that people who use local corn daily are likely to be particular about their diet in other respects, and often are in better financial condition than the average of the population. These associated factors would doubtless operate to diminish the incidence of pellagra in this group. On the other hand, certain families, after one of their members has contracted pellagra, not only tend to restrict their use of shipped corn, but in some cases they tend to substitute local corn for it. As we shall see in a subsequent section of this report, the members of such families seem to be especially likely to develop the disease, and this may be a factor in the high incidence in those using this food daily in certain villages. In any event, it is clear that this study of the use of local corn has failed to reveal any consistent relation between it and pellagra.

ANY CORN-MEAL

Corn-meal was used by the vast majority of the population studied. Most of those who never used shipped corn-meal did use local corn-meal. It therefore seems desirable to examine the data concerning the use of any corn-meal. The records in respect to this foodstuff include 858 families present in the six villages at the time of our census in 1913, of which 137 contained one or more cases of pellagra. The distribution of the 858 total families and of the 137 pellagrin families in respect to the use of corn-meal is shown in Tables 33, 34, 35 and 36.

TABLE 33.—Distribution of Total Families According to Frequency of Use of Any Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	63	26	13	0	0	1	2	105
W	67	24	3	0	0	0	0	94
P	137	59	27	0	0	0	2	225
Sa	59	26	14	0	0	0	1	100
A	40	23	19	1	0	0	0	83
Sp	149	49	29	2	1	0	21	251
Total..	515	207	105	3	1	1	26	858

TABLE 34.—Families Containing One or More Cases of Pellagra, Distributed According to Frequency of Use of Any Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	8	3	3	0	0	0	0	14
W	11	6	2	0	0	0	0	19
P	14	10	5	0	0	0	0	29
Sa	15	7	4	0	0	0	1	27
A	7	5	5	0	0	0	0	17
Sp	16	6	3	2	1	0	3	31
Total..	71	37	22	2	1	0	4	137

Corn-meal was used by all but 26 of the 858 families, and by 515 of these families it was used daily. In one village, Sp, there were 21 families who avoided the use of corn-meal, probably as a prophylactic measure against pellagra. In every one of the six villages the existence of pellagra was relatively less frequent in the families using corn-meal daily than in those using it rarely. This is probably due to a tendency on the part of some pellagrin families to restrict their use of corn-meal. It certainly does not indicate any consistent positive correlation between use of corn-meal and presence of pellagra in these families.

TABLE 35.—Summarized Distribution of Families According to the Use of Any Corn-Meal

Village	Total Families			Pellagrin Families		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	63	26	16	8	3	3
W	67	24	3	11	6	2
P	137	59	29	14	10	5
Sa	59	26	15	15	7	5
A	40	24	19	7	5	5
Sp	149	52	50	16	9	6
Total	515	211	132	71	40	26

TABLE 36.—Pellagrin Families in Total Families in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	12.7	11.5	18.8	13.3
W	16.4	25.0	66.7	20.2
P	10.2	16.9	17.2	12.9
Sa	25.4	26.9	33.3	27.0
A	17.5	20.8	26.3	20.5
Sp	10.7	17.3	12.0	12.4
Total.........	13.8	19.0	19.7	16.0

These 858 families included 5,095 individuals, of whom 196 were pellagrins. Their distribution in respect to the use of corn-meal is shown in Tables 37, 38, 39 and 40.

It is evident that nearly everybody in these villages used corn-meal to some extent. Only 123 persons were members of families in which this foodstuff was not used and 97 of these persons were in one village, Sp. The inverse relation between the existence of pellagra and the use of corn is clearly shown in Table 40. In every village there were relatively more cases of pellagra in the population using this food rarely or never than in the group using it daily. Of

TABLE 37.—Distribution of Total Individuals According to Frequency of Use of Any Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	381	141	70	0	0	2	6	600
W	415	128	17	0	0	0	0	560
P	850	377	147	0	0	0	11	1,385
Sa	367	183	77	0	0	0	9	636
A	212	152	86	4	0	0	0	454
Sp	910	278	159	9	7	0	97	1,460
Total..	3,135	1,259	556	13	7	2	123	5,095

TABLE 38.—Distribution of Pellagrins According to Frequency of Use of Any Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	12	3	5	0	0	0	0	25
W	14	8	3	0	0	0	0	38
P	18	12	8	0	0	0	1	38
Sa	20	12	5	0	0	0	0	27
A	12	7	8	0	0	0	5	46
Sp	22	9	6	3	1	0	0	20
Total..	98	51	35	3	1	0	6	194

TABLE 39.—Summarized Distribution According to Frequency of Use of Any Corn-Meal

Village	Total Population			Individual Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	381	141	78	12	3	5
W	415	128	17	14	8	3
P	850	377	158	18	12	8
Sa	367	183	86	20	12	6
A	212	156	86	12	7	8
Sp	910	294	256	22	13	11
Total	3,135	1,279	681	98	55	41

the 123 persons recorded as never using corn-meal, six (or 4.9 per cent.) were pellagrins, a relative morbidity greater than that observed in persons using corn-meal daily. It should be noted, however, that this group is made up of persons who were not using corn-meal at the time of the census and had not used it for the preceding two years. Doubtless most if not all these persons had at some time used corn-meal. The information in regard to diet in these tables refers therefore to the diet at the time of observation, namely, 1912 and 1913, and not necessarily to the diet at the time when pellagra originated.

TABLE 40.—Pellagra Morbidity in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	3.15	2.13	6.41	3.33
W	3.37	6.25	17.65	3.46
P	2.12	3.18	5.06	2.74
Sa	5.45	6.56	6.98	5.97
A	5.66	4.49	9.30	5.95
Sp	2.42	4.42	4.30	3.15
Total.........	3.13	4.30	6.02	3.81

TABLE 41.—Distribution of Total Families According to Frequency of Use of Any Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	65	27	13	0	0	1	2	108
W	68	24	4	0	0	0	1	97
P	137	59	27	0	0	0	2	225
Sa	61	26	14	0	0	0	1	102
A	40	23	19	1	0	0	0	83
Sp	150	50	29	2	1	0	21	253
Total..	521	209	106	3	1	1	27	868

The use of any corn-meal by incident cases of pellagra would appear to be of particular interest. The total population of these six mill villages, for which we have available data for a study of this topic, includes 868 families, in 85 of which new cases of pellagra developed in 1912 and 1913. The distribution of these families in respect to the use of any corn-meal is shown in Tables 41, 42, 43 and 44.

TABLE 42.—DISTRIBUTION OF FAMILIES IN WHICH NEW CASES OF PELLAGRA OCCURRED IN 1912 OR 1913, ACCORDING TO FREQUENCY OF USE OF ANY CORN-MEAL

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	10	4	2	0	0	0	0	16
W	7	1	1	0	0	0	1	10
P	11	4	5	0	0	0	0	20
Sa	6	4	2	0	0	0	0	12
A	3	4	3	0	0	0	0	10
Sp	10	4	1	0	0	0	2	17
Total..	47	21	14	0	0	0	3	85

TABLE 43.—SUMMARIZED DISTRIBUTION OF FAMILIES ACCORDING TO FREQUENCY OF USE OF ANY CORN-MEAL

Village	Total Families			Families with Incident Pellagra		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	65	27	16	10	4	2
W	68	24	5	7	1	2
P	137	59	29	11	4	5
Sa	61	26	15	6	4	2
A	40	24	19	3	4	3
Sp	150	53	50	10	4	3
Total	521	213	134	47	21	17

TABLE 44.—FAMILIES WITH INCIDENT PELLAGRA IN TOTAL FAMILIES IN EACH OF THE SUMMARIZED GROUPS, PER CENT.

Village	Daily	*Habitually*	*Rarely*	Total
I	15.4	14.8	12.5	14.8
W	10.3	4.2	40.0	10.3
P	8.0	6.8	17.2	8.9
Sa	9.8	15.4	13.3	11.8
A	7.5	16.7	15.8	12.0
Sp	6.7	7.5	6.0	6.7
Total.........	9.0	9.9	12.7	9.8

The incidence of new cases of pellagra in families shows no consistent relation to the frequency of use of corn-meal by these families. In four villages, W, P, Sa and A, and in the total families in the six villages, the families using corn-meal rarely or never developed new cases of pellagra relatively more frequently than those using this foodstuff daily, whereas in two villages, I and Sp, this relation was reversed. In the 27 families not using corn-meal during 1912 and 1913, there were three (or 11.1 per cent.) in which new cases of pellagra developed. This percentage is very similar to the analogous figures in the groups using corn-meal daily, habitually or rarely, and suggests that frequency of use of corn-meal in 1912 and 1913 had no direct relation to the development of new cases of pellagra in these families.

The 868 families just considered included 5,051 persons who were not pellagrins at the beginning of the year 1912. Of these 5,051 persons, 108 developed the disease during 1912 and 1913. The distribution of these persons according to the frequency of use of any corn-meal is shown in Tables 45, 46, 47 and 48.

TABLE 45.—Distribution of Individuals According to Frequency of Use of Any Corn-Meal

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	396	153	69	0	0	2	6	626
W	410	121	20	0	0	0	3	554
P	845	369	145	0	0	0	11	1,370
Sa	365	176	76	0	0	0	8	625
A	206	149	83	0	0	0	0	438
Sp	902	278	156	6	6	0	95	1,443
Total..	3,124	1,246	549	6	6	2	123	5,056

The incidence of new cases of pellagra was highest, in each of the six villages, among those persons using corn-meal rarely or never; and in the total population considered together, the incidence in this group was more than twice that of the group using corn-meal daily. The difference is more pronounced than that shown in the study of the families as units, and this means that families using little or no corn-meal not only developed new cases of pellagra more than the families using this food daily, but also developed multiple cases in the same family more frequently. The figures indicate very clearly that restriction or omission of corn-meal as an article of diet failed as

TABLE 46.—DISTRIBUTION OF INCIDENT PELLAGRINS ACCORDING TO FREQUENCY OF USE OF ANY CORN-MEAL

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	14	4	4	0	0	0	0	22
W	6	1	3	0	0	0	1	11
P	13	4	6	0	0	0	0	23
Sa	6	5	4	0	0	0	0	15
A	6	4	5	0	0	0	0	15
Sp	12	4	3	0	0	0	3	22
Total..	57	22	25	0	0	0	4	108

TABLE 47.—SUMMARIZED DISTRIBUTION ACCORDING TO FREQUENCY OF USE OF ANY CORN-MEAL

Village	Total Individuals			Incident Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	396	153	77	14	4	4
W	410	121	23	6	1	4
P	845	369	156	13	4	6
Sa	365	176	84	6	5	4
A	206	149	83	6	4	5
Sp	902	290	251	12	4	6
Total	3,124	1,258	674	57	22	29

TABLE 48.—INCIDENCE OF PELLAGRA IN EACH OF THE SUMMARIZED GROUPS, PER CENT.

Village	Daily	*Habitually*	*Rarely*	Total
I	3.54	2.61	5.19	3.51
W	1.46	0.83	17.39	1.99
P	1.54	1.08	3.85	1.68
Sa	1.64	2.84	4.76	2.40
A	2.91	2.68	6.02	3.42
Sp	1.33	1.38	2.39	1.52
Total.........	1.82	1.75	4.30	2.14

a prophylactic measure against pellagra in this population and suggest very strongly that it cannot be relied on for the control of pellagra.

The group of the population recorded as never using any corn-meal is of particular interest, and especially those families and the four persons in this group in whom pellagra developed in 1912 and 1913. One of these cases occurred in village W and the other three in village Sp, two of them in one family. The family in village W consisted of a man and wife, both about 65 years old, and three grown daughters. Corn-bread had been a staple article of diet until the summer of 1911, after which time it was no longer used. The youngest daughter, Case 78, developed pellagra in 1910 when she was 17 years old. The oldest daughter, Case 77, developed pellagra in the summer of 1911 when she was 27 years old. Both of these were pellagrins before the period of our observations. In June, 1912, about a year after the exclusion of corn-meal from the diet, the third sister, then aged 25, developed a typical erythema, and a diagnosis of pellagra was made. This recurred in July, 1913, without other symptoms. This case, 668, is regarded as incident in 1912, one year after the exclusion of corn-meal from the diet, and is therefore classed in the group of the population not using corn-meal. In the village Sp there are two families to be considered. One of these consisted of a mother, aged 41, and her son, aged 19. Corn-meal was not used, and our record clearly places this family in the class designated as never using this food. The record does not go into the matter in detail, and we do not know with certainty how long it had been excluded from the diet. The woman, Case 742, developed erythema for the first time in August, 1913, and this was accompanied by digestive disturbance and nervous symptoms. The remaining family consisted of a man and wife and three children. The wife, Case 25, developed pellagra in April, 1910, while residing at Columbia, S. C. She was then 38 years old. At that time she consulted one of the most eminent authorities on pellagra in this country and, at his advice, corn-meal was rigidly excluded from the diet of the whole family and none of it has been allowed in the house since 1910. The family moved to Spartanburg in the fall of 1911. The attack of pellagra recurred in the woman in 1911, 1912 and 1913. Early in June, 1912, two of the children, a girl aged 13 and a boy aged 6, developed typical erythema of the hands, and stomatitis. The boy also showed the eruption on his feet and had some diarrhea. In 1913 the boy remained free from symptoms of the disease, but it recurred in his sister with typical erythema, diarrhea and nervous excitability. These two children have been placed in the class not using corn-meal at the time of incidence of pellagra.

The theory that pellagra is caused by the excessive use of corn-meal is not supported by this study. Although every person in the population here considered probably ate corn-meal at some time in his life, there were found two cases of pellagra in which the use of this foodstuff could be excluded with almost absolute certainty for a period of two years preceding the development of the first sign of the disease, and there were two other cases in which corn-meal had

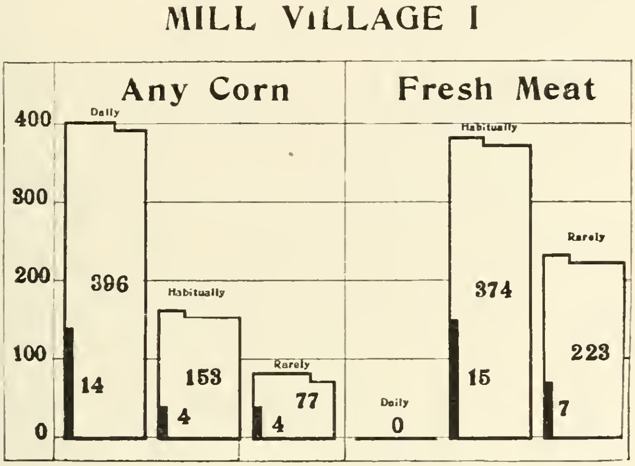


Fig. 9.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

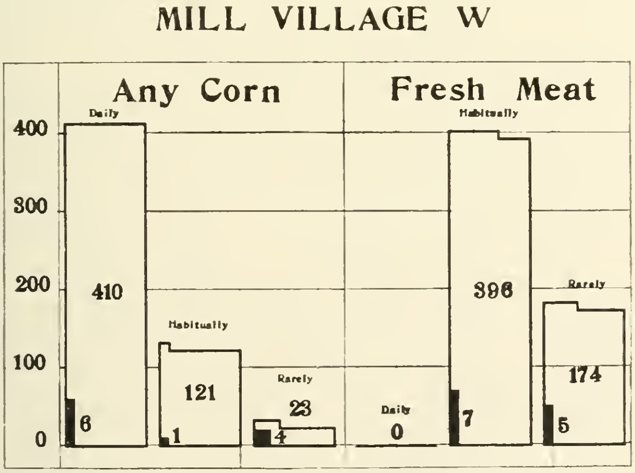


Fig. 10.—The area of each large white column indicattes the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

been excluded from the diet for a year previous to the development of the disease. The total persons in the population studied who have been classed as not using corn-meal amounted to only 123, and it may truly be said that non-pellagrins were placed in this group with much less careful scrutiny than were incident pellagrins. Of these 123 persons, four developed the disease in 1912 and 1913, an incidence of 3.25 per cent. This rate of incidence is greater than that observed among those using corn-meal daily (1.82 per cent.) or habitually (1.75 per cent.). In this population, therefore, the occurrence of new cases of pellagra seems to bear an inverse relation to the frequency of use of corn-meal.

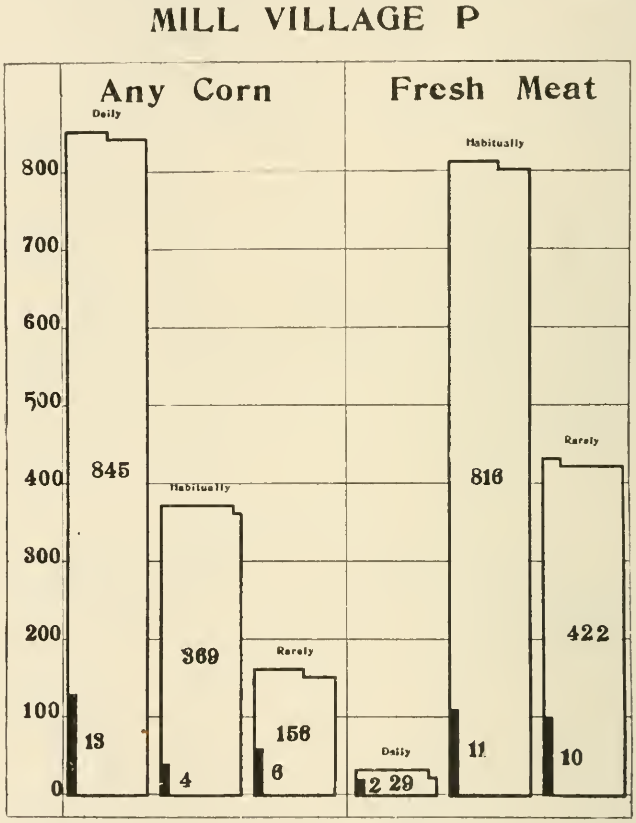


Fig. 11.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

Without committing ourselves to a final opinion, we may offer some suggestions in explanation of these facts. In the first place it seems certain that corn-meal cannot rightly be regarded as the essential cause of pellagra. The high incidence in those using little corn-meal we believe to be due to their close association with previous pellagra patients. It is chiefly in those families already having one case of pellagra that the use of this food has been intentionally restricted.

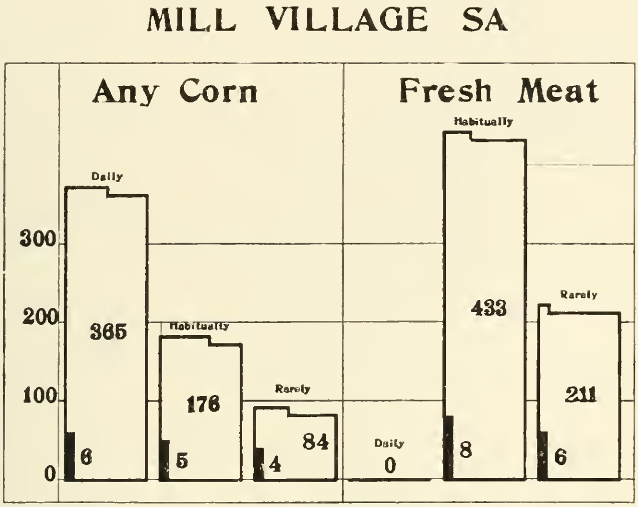


Fig. 12.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

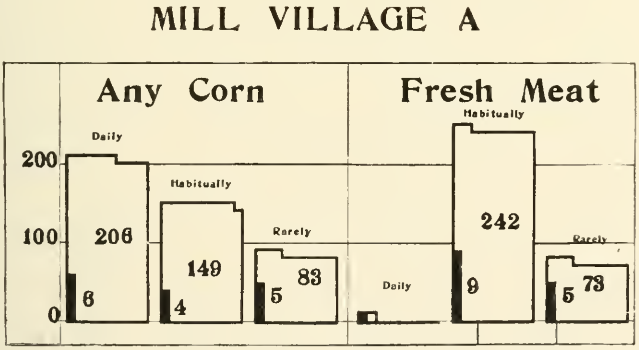


Fig. 13.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

This study has failed to reveal evidence of causal relationship of corn-meal to pellagra, and has shown that restriction of the use of corn-meal in the population studied has not proved effective as a prophylactic measure against pellagra.

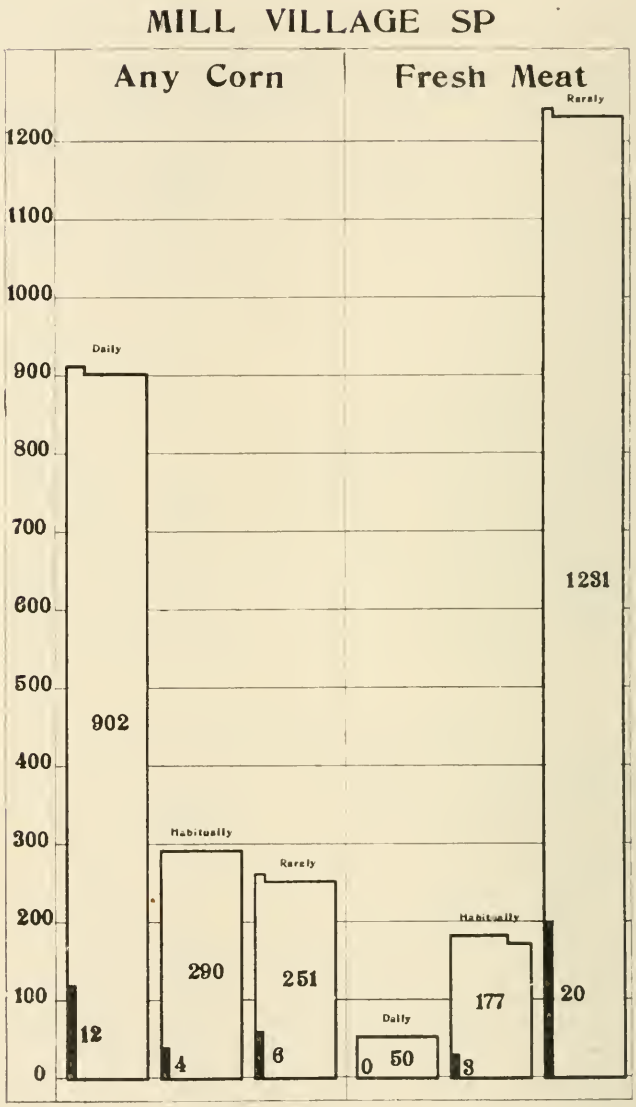


Fig. 14.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

FRESH MEATS

The recorded data in regard to the frequency of use of fresh meat by families present at the time of our census in these six mill villages relate to 861 families, of which 140 then contained one or more cases of pellagra. The distribution of these families according to frequency of use of this food is shown in Tables 49, 50, 51 and 52.

TABLE 49.—Distribution of Total Families According to Frequency of Use of Fresh Meat

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	14	33	2	40	4	2	95
W	0	6	7	2	52	15	12	94
P	4	88	47	1	65	13	12	230
Sa	0	8	31	0	62	6	0	107
A	1	14	15	2	33	7	11	83
Sp	8	21	204	1	9	0	9	252
Total..	13	151	337	8	261	45	46	861

TABLE 50.—Families Containing One or More Cases of Pellagra, Distributed According to Frequency of Use of Fresh Meat

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	1	4	0	8	1	0	14
W	0	1	3	0	10	2	3	19
P	1	12	6	0	7	3	1	30
Sa	0	4	9	0	15	1	0	29
A	1	4	4	0	8	0	0	17
Sp	1	1	26	1	2	0	0	31
Total..	3	23	52	1	50	7	4	140

The daily use of fresh meat was rather uncommon in this population, only thirteen families falling in this group. Almost half the families (420) used this food habitually, part time daily or part time habitually, and about half of them (428) used it rarely or never. In two of the villages we failed to find any families in which fresh meat was used daily. The families containing cases of pellagra occur in all the groups and are relatively most numerous in the group using fresh meat daily and least numerous in the summarized group using this food rarely or never. In those families avoiding fresh meat altogether, only 4 out of a total of 46 had cases of pellagra, or 8.7 per cent., a lower percentage than in any other group.

TABLE 51.—Summarized Distribution of Families According to Frequency of Use of Fresh Meat

Village	Total Families			Pellagrin Families		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	0	56	39	0	9	5
W	0	60	34	0	11	8
P	4	154	72	1	19	10
Sa	0	70	37	0	19	10
A	1	49	33	1	12	4
Sp	8	31	213	1	4	26
Total	13	420	428	3	74	63

TABLE 52.—Pellagrin Families in Total Families in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	....	16.1	12.8	14.7
W	....	18.3	23.5	20.2
P	25.0	12.3	13.9	13.0
Sa	....	27.1	27.0	27.1
A	100.0	24.5	12.1	20.5
Sp	12.5	12.9	12.2	12.3
Total.........	23.1	17.6	14.7	16.3

The 861 families just considered included 5,133 persons, of whom 199 were pellagrins. The distribution of these persons according to the frequency of use of fresh meat in their families is shown in Tables 53, 54, 55 and 56.

It is evident that only a few (82) persons in this population used fresh meat daily, while 263 did not use it at all. About half the population (2,591 persons) used fresh meat as often as twice a week during part of the year at least, while the remaining population used this food less frequently. In certain mill villages, I, W, Sa and A, fresh meat could be had at local markets in the village only during the colder season of the year; during the summer the inhabitants were com-

TABLE 53.—Distribution of Total Individuals According to Frequency of Use of Fresh Meat

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	102	182	13	233	28	11	569
W	0	43	43	16	339	70	64	575
P	29	550	281	4	403	73	71	1,411
Sa	0	62	183	0	375	32	0	652
A	2	88	75	23	163	32	67	450
Sp	51	115	1,198	2	60	0	50	1,476
Total..	82	960	1,962	58	1,573	235	263	5,133

TABLE 54.—Distribution of Pellagrins According to Frequency of Use of Fresh Meat

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	2	7	0	12	1	0	22
W	0	2	6	0	11	2	3	24
P	2	15	10	0	8	3	1	39
Sa	0	6	10	0	18	4	0	38
A	1	8	7	0	11	0	0	27
Sp	1	1	44	1	2	0	0	49
Total..	4	34	84	1	62	10	4	199

TABLE 55.—Summarized Distribution According to Frequency of Use of Fresh Meat

Village	Total Population			Individual Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	0	348	221	0	14	8
W	0	398	177	0	13	11
P	29	957	425	2	23	14
Sa	0	437	215	0	24	14
A	2	274	174	1	19	7
Sp	51	177	1,248	1	4	44
Total	82	2,591	2,460	4	97	98

pelled to get their meat at more distant markets. In the large villages, P and Sp, the local markets sold fresh meat throughout the year. It was in these two villages that the lowest morbidity from pellagra was observed, namely, 2.76 and 3.32 per cent.

TABLE 56.—Pellagra Morbidity in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	....	4.02	3.62	3.87
W	....	3.27	6.21	4.17
P	6.90	2.41	3.29	2.76
Sa	....	5.49	6.51	5.83
A	50.00	6.93	4.02	6.00
Sp	1.96	2.26	3.53	3.32
Total.........	4.88	3.74	3.98	3.88

When we turn our attention to the morbidity in each of the dietary groups, the relation of pellagra to the use of fresh meat is much less evident. In one village, Sp, the morbidity increases progressively with the decrease in frequency of use of fresh meat, daily 1.96, habitually 2.26, rarely 3.53 per cent., but in the same village there were 50 persons who did not use this food at all and none of them had pellagra. In the other villages the correlation is even more confused, and in the total population of the six villages the pellagra morbidity is actually highest in the group of 82 persons who used fresh meat daily, namely, 4.88 per cent., while of the 263 persons who never used it, only 4 suffered from the disease (1.52 per cent.). The recorded data do not seem, therefore, to indicate any consistent correlation between the existence of pellagra and deficiency of fresh meat in the dietary of this population.

The population to be considered in relation to incident cases of pellagra included 884 families, in 85 of which new cases of pellagra developed in 1912 and 1913. The distribution of these families according to frequency of use of fresh meat is shown in Tables 57, 58, 59 and 60.

There is not a consistent correlation between the frequency of use of fresh meat and the origin of new cases of pellagra in these families. In two villages, Sa and Sp, the incidence is highest in the summarized group using fresh meat rarely, but in three villages, I, W and A, it is lowest in this group. In the total families the occurrence of new cases of pellagra seems to be correlated directly with the frequency of use of fresh meat. Even in those 56 families never

TABLE 57.—DISTRIBUTION OF TOTAL FAMILIES ACCORDING TO FREQUENCY OF USE OF FRESH MEAT

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	17	34	2	40	4	12	109
W	0	7	7	2	53	16	12	97
P	4	88	47	1	65	13	12	230
Sa	0	9	31	0	63	6	0	109
A	1	15	15	2	33	7	11	84
Sp	8	22	206	1	9	0	9	255
Total..	13	158	340	8	263	46	56	884

TABLE 58.—DISTRIBUTION OF FAMILIES IN WHICH NEW CASES OF PELLAGRA OCCURRED IN 1912 OR 1913, ACCORDING TO FREQUENCY OF USE OF FRESH MEAT

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	4	5	0	6	1	0	16
W	0	2	1	0	6	1	1	11
P	1	7	6	0	4	2	0	20
Sa	0	2	4	0	5	0	0	11
A	1	5	2	0	2	0	0	10
Sp	0	1	15	1	0	0	0	17
Total..	2	21	33	1	23	4	1	85

TABLE 59.—SUMMARIZED DISTRIBUTION OF FAMILIES ACCORDING TO FREQUENCY OF USE OF FRESH MEAT

Village	Total Families			Families with Incident Pellagra		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	0	59	50	0	10	6
W	0	62	35	0	8	3
P	4	154	72	1	11	8
Sa	0	72	37	0	7	4
A	1	50	33	1	7	2
Sp	8	32	215	0	2	15
Total	13	429	442	2	45	38

using fresh meat, there was only one in which an incident case of pellagra appeared, or 1.8 per cent., a very much lower rate of incidence than was observed in any other group.

In the 884 families just considered, there were 4,853 persons, of whom 109 contracted pellagra in 1912 and 1913. The distribution of these persons according to the frequency of use of fresh meat in their families is shown in Tables 61, 62, 63 and 64.

TABLE 60.—Families with Incident Pellagra in Total Families in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	....	16.9	12.0	14.7
W	....	12.9	8.6	11.3
P	25.0	7.1	11.1	8.7
Sa	....	9.7	10.8	10.1
A	100.0	14.0	6.1	11.9
Sp	0.0	6.3	7.0	6.7
Total.........	15.4	10.5	8.6	9.6

TABLE 61.—Distribution of Individuals According to Frequency of Use of Fresh Meat

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	131	184	13	230	28	11	597
W	0	45	38	16	335	74	62	570
P	29	464	280	4	348	72	70	1,267
Sa	0	65	179	0	368	32	0	644
A	2	88	73	0	154	0	0	317
Sp	50	117	1,181	2	58	0	50	1,458
Total..	81	910	1,935	35	1,493	206	193	4,853

New cases of pellagra developed in all the groups, and there is no consistent correlation between the incidence of these cases and the frequenty of use of fresh meat. Of the 81 persons in families using this food daily, 3 acquired pellagra during the two years, a higher incidence (3.70 per cent.) than was observed in any other group. In the 193 persons who did not use fresh meat at all, only 1 new case of pellagra was observed, an incidence of 0.52 per cent., much lower than in any other group. In fact, in the village in which this one case

TABLE 62.—Distribution of Incident Pellagrins According to Frequency of Use of Fresh Meat

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	6	6	0	9	1	0	22
W	0	2	1	0	5	3	1	12
P	2	7	8	0	4	2	0	23
Sa	0	3	6	0	5	0	0	14
A	1	7	5	0	2	0	0	15
Sp	0	2	20	1	0	0	0	23
Total..	3	27	46	1	25	6	1	109

TABLE 63.—Summarized Distribution According to Frequency of Use of Fresh Meat

Village	Total Population			Incident Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	0	374	223	0	15	7
W	0	396	174	0	7	5
P	29	816	422	2	11	10
Sa	0	433	211	0	8	6
A	2	242	73	1	9	5
Sp	50	177	1,231	0	3	20
Total	81	2,438	2,334	3	53	53

TABLE 64.—Incidence of Pellagra in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	....	4.01	3.14	3.69
W	....	1.77	2.87	2.11
P	6.90	1.35	2.37	1.82
Sa	....	1.85	2.84	2.17
A	50.00	3.72	6.85	4.73
Sp	0.00	1.69	1.62	1.58
Total.........	3.70	2.17	2.27	2.25

occurred there were 62 persons who did not use fresh meat, so that the incidence for that village alone was only 1.61 per cent. for this group. One finds here no support for the idea that deficiency of fresh meat in the diet is a cause of pellagra. On the contrary, it would appear quite certain that in the population here studied those avoiding fresh meat contracted this disease least.

TABLE 65.—Distribution of Total Families According to Frequency of Use of Canned Goods

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	2	17	58	0	0	0	10	87
W	3	27	48	1	0	0	12	91
P	4	58	136	0	0	1	27	226
Sa	1	21	75	1	0	0	8	106
A	3	21	54	0	0	0	3	81
Sp	3	15	178	0	0	0	54	250
Total..	16	159	549	2	0	1	114	841

TABLE 66.—Families Containing One or More Cases of Pellagra Distributed According to Frequency of Use of Canned Goods

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	4	9	0	0	0	0	13
W	0	1	12	1	0	0	3	17
P	0	9	16	0	0	1	5	31
Sa	0	6	20	1	0	0	2	29
A	0	3	14	0	0	0	0	17
Sp	0	0	24	0	0	0	7	31
Total..	0	23	95	2	0	1	17	138

CANNED GOODS

The data in regard to the use of canned goods (tinned foods) include 841 families present at the time of census, 138 of which contained one or more cases of pellagra at that time. The distribution of these families according to frequency of use of tinned foods is shown in Tables 65, 66, 67 and 68.

There is evidently no consistent positive correlation between the frequency of use of canned goods and the existence of cases of pellagra in these families. In fact the indication, if anything, would appear to be the reverse. The 16 families using this kind of food daily had no pellagra, while, of the 114 families avoiding canned goods altogether, no less than seventeen (14.9 per cent.), contained one or more cases of the disease.

TABLE 67.—Summarized Distribution of Families According to Frequency of Use of Canned Goods

Village	Total Families			Pellagrin Families		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	2	17	68	0	4	9
W	3	28	60	0	2	15
P	4	58	164	0	9	22
Sa	1	22	83	0	7	22
A	3	21	57	0	3	14
Sp	3	15	232	0	0	31
Total	16	161	664	0	25	113

TABLE 68.—Pellagrin Families in Total Families in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	0.0	23.5	13.2	14.9
W	0.0	7.1	25.0	18.7
P	0.0	15.5	13.4	13.7
Sa	0.0	31.8	26.5	27.4
A	0.0	14.3	24.6	21.0
Sp	0.0	0.0	13.4	12.4
Total.........	0.0	15.5	17.0	16.4

The 841 families just considered contained 4,966 persons, of whom 192 were pellagrins. The distribution of these persons according to the frequency of use of tinned foods in their families is shown in Tables 69, 70, 71 and 72.

Evidence of correlation between the existence of pellagra in a family and the frequency of use of canned goods is not to be found in these tables. The 89 persons using canned foods daily had no pellagra. In the 643 persons who avoided such foods, 18, or 2.80 per cent., were pellagrins.

TABLE 69.—Distribution of Total Individuals According to Frequency of Use of Canned Goods

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	9	111	337	0	0	0	60	517
W	12	168	292	3	0	0	71	546
P	24	416	788	0	0	5	148	1,381
Sa	2	141	462	5	0	0	32	642
A	20	114	286	0	0	0	11	431
Sp	22	88	1,018	0	0	0	321	1,449
Total..	89	1,038	3,183	8	0	5	643	4,966

TABLE 70.—Distribution of Pellagrins According to Frequency of Use of Canned Goods

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	5	14	0	0	0	0	19
W	0	1	17	1	0	0	3	22
P	0	12	21	0	0	1	6	40
Sa	0	9	25	2	0	0	2	38
A	0	4	22	0	0	0	0	26
Sp	0	0	40	0	0	0	7	47
Total..	0	31	139	3	0	1	18	192

TABLE 71.—Summarized Distribution According to Frequency of Use of Canned Goods

Village	Total Population			Individual Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	9	111	397	0	5	14
W	12	171	363	0	2	20
P	24	416	941	0	12	28
Sa	2	146	494	0	11	27
A	20	114	297	0	4	22
Sp	22	88	1,339	0	0	47
Total	89	1,046	3,831	0	34	158

For the study of the occurrence of new cases of pellagra in relation to the use of canned goods, we have data on 853 families, in 85 of which one or more new cases of the disease developed in 1912 or 1913. The distribution of these families in respect to frequency of use of tinned foods is shown in Tables 73, 74, 75 and 76.

TABLE 72.—Pellagra Morbidity in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	0	4.55	3.53	3.68
W	0	1.17	5.51	4.03
P	0	2.88	2.98	2.90
Sa	0	7.53	5.47	5.92
A	0	3.51	7.41	6.03
Sp	0	0.00	3.51	3.24
Total.........	0	3.25	4.12	3.87

TABLE 73.—Distribution of Total Families According to Frequency of Use of Canned Goods

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	2	17	61	0	1	0	10	91
W	3	27	50	1	0	0	13	94
P	4	58	136	0	0	1	27	226
Sa	1	22	76	1	1	0	8	109
A	3	21	54	0	0	0	3	81
Sp	3	15	180	0	0	0	54	252
Total..	16	160	557	2	2	1	115	853

The occurrence of new cases of pellagra in a family does not appear to have been influenced in any consistent way by the frequency with which the family used canned goods. The 16 families using canned goods daily had no new cases of the disease during the two years. Of the 115 families avoiding the use of these foods, 8 (7 per cent.), had new cases of pellagra during 1912 or 1913.

The 853 families just considered included 4,874 persons, of whom 109 persons contracted pellagra in 1912 or 1913. The distribution of these persons according to frequency of use of tinned foods is shown in Tables 77, 78, 79 and 80.

TABLE 74.—Distribution of Families in which New Cases of Pellagra Occurred in 1912 or 1913, According to Frequency of Use of Canned Goods

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	2	12	0	1	0	0	15
W	0	0	9	1	0	0	1	11
P	0	8	10	0	0	1	1	20
Sa	0	2	8	1	1	0	0	12
A	0	2	8	0	0	0	0	10
Sp	0	0	11	0	0	0	6	17
Total..	0	14	58	2	2	1	8	85

TABLE 75.—Summarized Distribution of Families According to Frequency of Use of Canned Goods

Village	Total Families			Families with Incident Pellagra		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	2	18	71	0	3	12
W	3	28	63	0	1	10
P	4	58	164	0	8	12
Sa	1	24	84	0	4	8
A	3	21	57	0	2	8
Sp	3	15	234	0	0	17
Total	16	164	673	0	18	67

TABLE 76.—Families with Incident Pellagra in Total Families in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	0	16.7	16.9	16.5
W	0	3.6	15.9	11.7
P	0	13.8	7.3	8.8
Sa	0	16.7	9.5	11.0
A	0	9.5	14.0	12.3
Sp	0	0.0	7.3	6.7
Total.........	0	11.0	10.0	10.0

TABLE 77.—DISTRIBUTION OF INDIVIDUALS ACCORDING TO FREQUENCY OF USE OF CANNED GOODS

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	9	108	367	0	3	0	60	547
W	12	168	291	3	0	0	71	545
P	24	352	778	0	0	5	144	1,303
Sa	2	140	452	5	7	0	32	638
A	20	112	277	0	0	0	0	409
Sp	22	88	1,002	0	0	0	320	1,432
Total..	89	968	3,167	8	10	5	627	4,874

TABLE 78.—DISTRIBUTION OF INCIDENT PELLAGRINS ACCORDING TO FREQUENCY OF USE OF CANNED GOODS

Village	Daily	Habitually	Rarely	Part Time			Never	Total
				Daily	Habitually	Rarely		
I	0	2	18	0	1	0	0	21
W	0	0	11	1	0	0	1	13
P	0	9	11	0	0	1	2	23
Sa	0	2	9	2	1	0	0	14
A	0	2	13	0	0	0	0	15
Sp	0	0	17	0	0	0	6	23
Total..	0	15	79	3	2	1	9	109

TABLE 79.—SUMMARIZED DISTRIBUTION ACCORDING TO FREQUENCY OF USE OF CANNED GOODS

Village	Total Individuals			Incident Pellagrins		
	Daily	*Habitually*	*Rarely*	Daily	*Habitually*	*Rarely*
I	9	111	427	0	3	18
W	12	171	362	0	1	12
P	24	352	927	0	9	14
Sa	2	152	484	0	5	9
A	20	112	277	0	2	13
Sp	22	88	1,322	0	0	23
Total	89	986	3,799	0	20	89

TABLE 80.—Incidence of Pellagra in Each of the Summarized Groups, Per Cent.

Village	Daily	*Habitually*	*Rarely*	Total
I	0	2.73	4.22	3.84
W	0	5.85	3.31	2.39
P	0	2.56	1.51	1.77
Sa	0	3.29	1.86	2.19
A	0	1.79	4.69	3.67
Sp	0	0.00	1.74	1.61
Total.........	0	2.03	2.34	2.24

There is no evident consistent correlation discovered here between the use of canned goods and the development of new cases of pellagra. The 89 persons in families using these foods daily remained free from pellagra. Of the 627 persons who avoided altogether the use of canned goods, 9, or 1.44 per cent., developed the disease in the course of the two years 1912 and 1913. There is no significant difference in incidence of pellagra between the groups using these foods habitually and the groups using them rarely. This study has failed therefore to discover any evidence that the use of canned goods causes pellagra, and suggests that these foods had no part in the causation of this disease in this particular population.

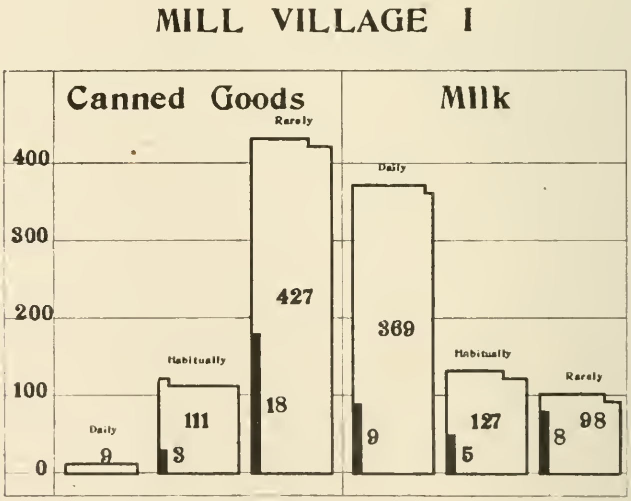


Fig. 15.—The area of each large white column indicates the number of nonpellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

MILL VILLAGE W

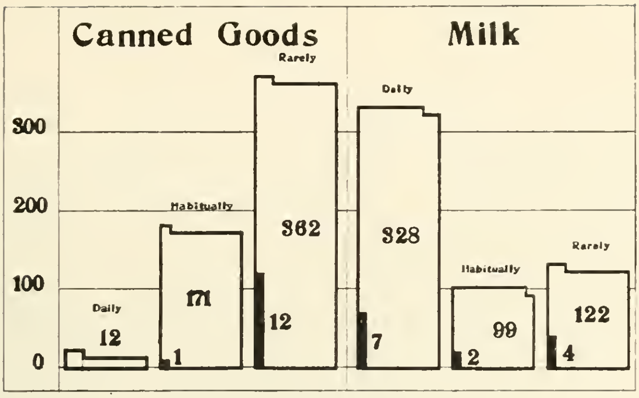


Fig. 16.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

MILL VILLAGE P

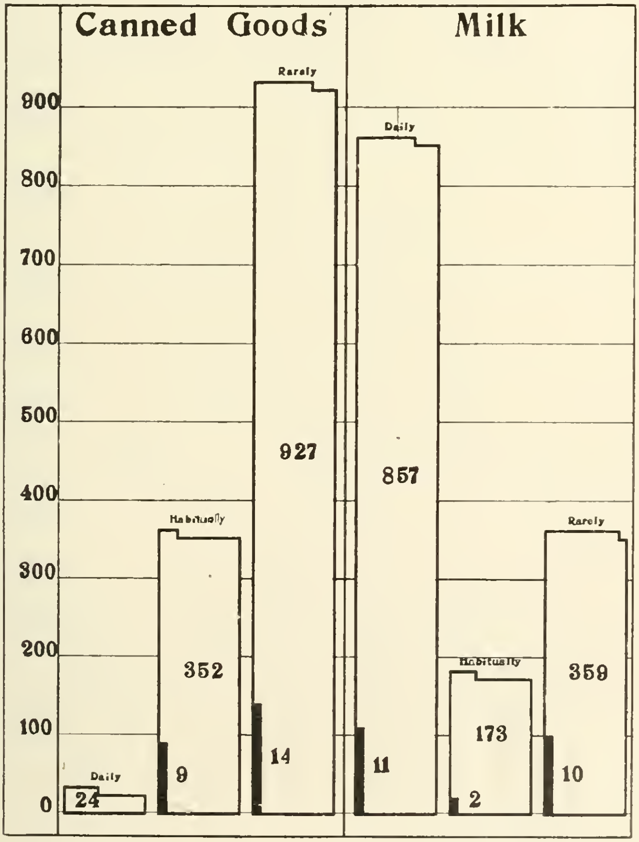


Fig. 17.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

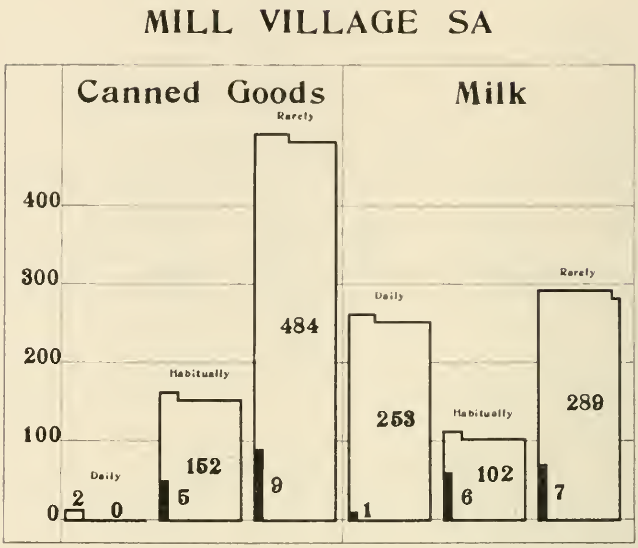


Fig. 18.—The area of each large white column indicates the number of nonpellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

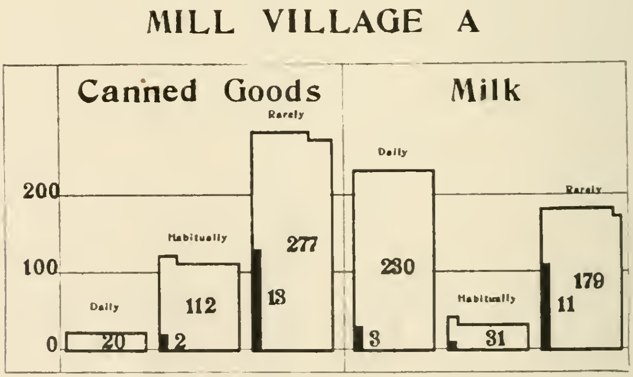


Fig. 19.—The area of each large white column indicates the number of nonpellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

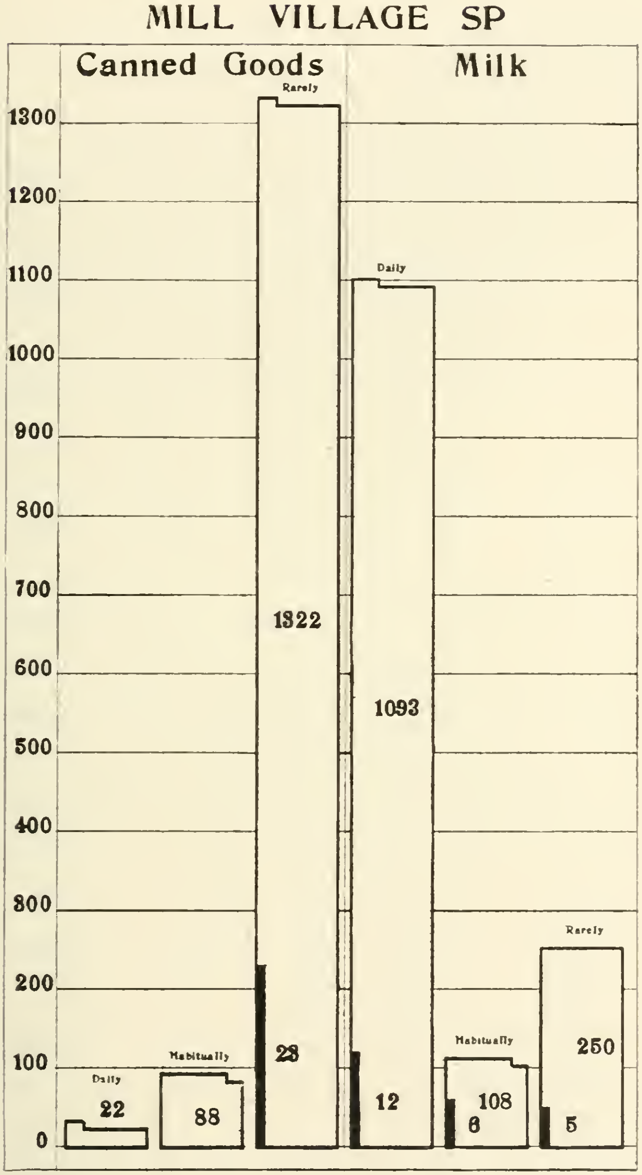


Fig. 20.—The area of each large white column indicates the number of non-pellagrin individuals using the food daily, *habitually* or *rarely*. The included black column indicates the portion of the respective group which contracted pellagra in 1912 or 1913.

MILK

Of the six mill villages used for the detailed study of the foods, only one, Sp, had a market dairy. In the other five villages the families using milk daily kept their own cows, as a rule. In only a few instances was milk purchased daily from neighboring families. Those who used milk less frequently did not possess cows. In many families using milk daily, this food was taken as a beverage in the form of buttermilk. Drinking of sweet milk was very exceptional.

TABLE 81.—Distribution of Families According to Frequency of Use of Milk

Village	Total Families					Pellagrin Families				
	Daily	Habitually	Rarely	Never	Total	Daily	Habitually	Rarely	Never	Total
I	63	21	10	2	96	9	3	0	2	14
W	53	17	8	14	92	7	5	1	6	19
P	137	32	40*	21	230	16	6	5*	4	31
Sa	46	16	18	27	107	13	5	4	7	29
A	39	7†	13	24	83	6	3†	4	4	17
Sp	187	18	29	22	256	20	3	2	6	31
Total	525	111	118	110	864	71	25	16	29	141

* Includes one family using milk part time rarely. † Includes one family using milk part time habitually.

TABLE 82.—Pellagrin Families in Total Families in Each of the Groups, Per Cent.

Village	Daily	Habitually	Rarely	Never	Rarely or Never*	Total
I	14.3	14.3	0.0	100.0	16.7	14.6
W	13.2	29.4	12.5	42.9	31.8	20.7
P	11.7	18.8	12.5	19.0	14.8	13.5
Sa	28.3	31.3	22.2	25.9	24.4	27.1
A	15.4	42.9	30.8	16.7	21.6	20.5
Sp	10.7	16.7	6.9	27.3	15.7	12.1
Total	13.5	22.5	13.6	26.4	19.7	16.3

* This group corresponds to the one designated as *Rarely* in the preceding summary tables.

The recorded data concerning the population present at the time of our census relate to 864 families, in 141 of which one or more cases of pellagra existed at the time. The distribution of these families in respect to the frequency of use of milk is shown in Tables 81 and 82. Only one family used milk part time habitually; one, part time rarely, and none part time daily. These groups are therefore not given separate consideration in the tables.

Milk was used daily by considerably more than half the total families; and in only two of the villages, Sa and A, was this food daily used by less than half the population. The groups using milk habitually, rarely and never are well represented, each including more than 100 families. Table 82 (of percentages) suggests that pellagra was, on the whole, somewhat less common in families using milk daily. Furthermore, the 110 families avoiding altogether the use of milk included 29, or 26.4 per cent., in which pellagra existed, a figure distinctly above the average for the total families considered.

TABLE 83.—Distribution According to Frequency of Use of Milk

Village	Total Populattion					Individual Pellagrins				
	Daily	Habitually	Rarely	Never	Total	Daily	Habitually	Rarely	Never	Total
I	371	127	53	13	564	11	5	0	4	20
W	327	102	55	69	553	9	7	1	7	24
P	865	177	233*	134	1,409	19	6	7†	8	40
Sa	268	103	126	159	656	16	7	7	8	38
A	236	40‡	46	129	451	9	6§	4	8	27
Sp	1,104	104	144	115	1,467	25	7	6	8	46
Total	3,171	653	657	619	5,100	89	38	25	43	195

* Includes five persons using milk part time rarely. † Includes one person using milk part time rarely. ‡ Includes eight persons using milk part time habitually. § Includes one person using milk part time habitually.

TABLE 84.—Pellagra Morbidity in Each of the Groups, Per Cent.

Village	Daily	Habitually	Rarely	Never	Rarely or Never*	Total
I	2.96	3.94	0.00	3.08	6.06	3.55
W	2.75	6.86	1.82	10.14	6.45	4.34
P	2.20	3.39	3.00	5.97	4.09	2.84
Sa	5.97	6.80	5.56	5.03	5.26	5.79
A	3.81	15.00	8.70	6.20	6.86	5.99
Sp	2.26	6.73	4.17	6.96	5.41	3.14
Total	2.81	5.82	3.81	6.95	5.33	3.82

* This group corresponds to the one designated as *Rarely* in the preceding summary tables.

The 864 families just considered included 5,100 persons, of whom 195 were pellagrins at the time of our census. Inasmuch as the four groups using milk daily, habitually, rarely and never each contain more than 600 individuals, while the other groups are very small, it seems best to present the figures in four groups (Tables 83 and 84).

The correlation between frequency of use of milk and freedom from pellagra is not definitely consistent; but, in five of the villages, those avoiding the use of milk altogether had relatively more pellagra than those using this food daily. The figures for the total population also show a marked difference, 2.81 per cent. of those using milk daily being pellagrins against 6.95 per cent. of those never using milk. It would appear, therefore, that pellagrins used milk somewhat less frequently, on the whole, than the population which was free from this disease.

For the study of the relation of milk in the diet to the origin of new cases of pellagra, we have records of 877 families of which 86 developed one or more incident cases of the disease in 1912 or 1913. There were no families in the groups "Part Time Daily" and "Part Time Habitually," and only one family of five persons in the group "Part Time Rarely." It therefore seems unneccessary to present the usual detailed tables of the seven groups. In Tables 85 and 86 the one family of five persons using milk part time rarely has been classed with those using milk rarely. One new case of pellagra developed in this family.

TABLE 85.—Distribution of Families According to Frequency of Use of Milk

Village	Total Families					Families with Incident Pellagra				
	Daily	Habitually	Rarely	Never	Total	Daily	Habitually	Rarely	Never	Total
I	63	21	11	5	100	8	3	1	4	16
W	54	18	9	14	95	5	2	1	3	11
P	137	32	40*	23	232	10	2	2*	6	20
Sa	46	16	19	28	109	1	5	2	4	12
A	39	6	13	25	83	3	1	1	5	10
Sp	188	19	29	22	258	9	4	2	2	17
Total	527	112	121	117	877	36	17	9	24	86

* Includes one family using milk rarely for part of the year only.

In Table 86 the group designated "Rarely or Never" is strictly comparable with the summarized group designated as *Rarely* in the presentation of data concerning other foods. There is a fairly consistent correlation shown in this table, indicating that the families in which milk was not used were the ones in which new cases of pellagra most commonly appeared, while, on the other hand, those families using this food daily developed new cases the most infrequently. This difference would appear to have some significance.

The 877 families just considered included 5,067 persons free from pellagra at the beginning of 1912. Of these 5,067 persons, 110 contracted the disease during 1912 and 1913. One family of five persons used milk rarely for part of the year only. All the other persons fell strictly within the groups shown in Tables 87 and 88.

TABLE 86.—FAMILIES WITH INCIDENT PELLAGRA IN TOTAL FAMILIES IN EACH OF THE GROUPS, PER CENT.

Village	Daily	Habitually	Rarely	Never	Rarely or Never	Total
I	12.7	14.3	9.1	80.0	31.3	16.0
W	9.3	11.1	11.1	21.4	17.4	11.6
P	7.3	6.3	5.0	26.1	12.7	8.6
Sa	2.2	31.3	10.5	14.3	12.8	11.0
A	7.7	16.7	7.7	20.0	15.8	12.0
Sp	4.8	21.1	6.9	9.1	7.8	6.6
Total	6.8	15.2	7.4	20.5	13.9	9.8

TABLE 87.—DISTRIBUTION ACCORDING TO FREQUENCY OF USE OF MILK

Village	Total Individuals					Incident Pellagrins				
	Daily	Habitually	Rarely	Never	Total	Daily	Habitually	Rarely	Never	Total
I	369	127	56	42	594	9	5	1	7	22
W	328	99	57	65	549	7	2	1	3	13
P	857	173	228*	131	1,389	11	2	2†	8	23
Sa	253	102	127	162	644	1	6	2	5	14
A	230	31	43	136	440	3	1	1	10	15
Sp	1,093	108	141	109	1,451	12	6	3	2	23
Total	3,130	640	652	645	5,067	43	22	10	35	110

* Includes five persons using milk part time rarely. † Includes one person using milk part time rarely.

TABLE 88.—INCIDENCE OF PELLAGRA IN EACH OF THE GROUPS, PER CENT.

Village	Daily	Habitually	Rarely	Never	Rarely or Never	Total
I	2.44	3.94	1.79	16.67	8.16	3.70
W	2.13	2.02	1.75	4.62	3.28	2.37
P	1.28	1.16	0.88	6.11	2.79	1.66
Sa	0.40	5.88	1.57	3.09	2.42	2.17
A	1.30	3.23	2.33	7.35	6.15	3.41
Sp	1.09	5.56	2.13	1.84	2.00	1.59
Total	1.37	3.44	1.53	5.43	3.47	2.17

It is evident that in the whole population those persons using milk daily contracted pellagra the least. Of the 3,130 persons in this group, only 43 acquired the disease during the two years. In the whole population also, it is clear that those avoiding the use of milk suffered most from the development of new cases of pellagra. The incidence of the disease in the latter group, 5.43 per cent., is nearly four times that in the former, 1.37 per cent. In every one of the six villages the group using milk daily showed a lower incidence than the average for that village, and those never using milk showed a higher incidence than the average. The correlation is quite inconsistent in the groups using milk habitually and rarely. The tendency toward correlation between the occurrence of new cases of pellagra and the deficiency of milk in the diet is nevertheless distinctly evident, on the whole, and suggests that the use of milk (including buttermilk) as a food has some value in the prevention of pellagra.

TABLE 89.—Distribution of Families According to Frequency of Use of Eggs

Village	Total Families					Pellagrin Families				
	Daily	Habitually	Rarely	Never	Total	Daily	Habitually	Rarely	Never	Total
I	37	42	13	0	92	3	6	2	0	11
W	14	52	23	6	95	3	7	8	1	19
P	35	125	55	9	224	3	17	5	1	26
Sa	26	46	31	3	106	10	9	9	1	29
A	13	35*	30	5	83	1	8*	8	0	17
Sp	28	90	129	5	252	2	14	14	1	31
Total	153	390	281	28	852	22	61	46	4	133

* Includes one family using eggs part time habitually.

EGGS

The recorded data concerning the use of eggs relate to 852 families present in the six villages at the time of our census, of which 133 contained one or more pellagrins. The distribution of these families according to the frequency of use of eggs is shown in Tables 89 and 90.

The tabulated data show very clearly that there was no significant variation in existence of pellagra in families correlated with a difference in frequency of use of eggs. The percentage of families with pellagra in the different groups is remarkably uniform.

The 852 families contained 5,068 persons, of whom 186 were pellagrins at the time of our census. The distribution of these persons according to frequency of use of eggs in their families, is shown in Tables 91 and 92.

TABLE 90.—PELLAGRIN FAMILIES IN TOTAL FAMILIES IN EACH OF THE GROUPS, PER CENT.

Village	Daily	Habitually	Rarely	Never	Rarely or Never	Total
I	8.1	14.3	15.4	....	15.4	12.0
W	21.4	13.5	34.8	16.7	31.0	20.0
P	8.6	13.6	9.1	11.1	9.4	11.6
Sa	38.5	19.6	29.0	33.3	29.4	27.4
A	7.7	22.9	26.7	0.0	22.9	20.5
Sp	7.1	15.6	10.9	20.0	11.2	12.3
Total	14.4	15.6	16.4	14.3	16.2	15.6

TABLE 91.—DISTRIBUTION ACCORDING TO FREQUENCY OF USE OF EGGS

Village	Total Population					Individual Pellagrins				
	Daily	Habitually	Rarely	Never	Total	Daily	Habitually	Rarely	Never	Total
I	225	253	64	0	542	3	9	4	0	16
W	75	317	144	32	568	5	8	10	1	24
P	198	786	337	62	1,383	3	22	8	1	34
Sa	157	267	209	19	652	15	9	13	1	38
A	65	187*	169	28	449	1	16†	10	0	27
Sp	151	505	789	29	1,474	3	15	27	2	47
Total	871	2,315	1,712	170	5,068	30	79	72	5	186

* Includes eight persons using eggs part time habitually. † Includes four persons using eggs part time habitually.

TABLE 92.—PELLAGRA MORBIDITY IN EACH OF THE GROUPS, PER CENT.

Village	Daily	Habitually	Rarely	Never	Rarely or Never	Total
I	1.33	3.56	6.25	....	6.25	2.95
W	6.67	2.52	6.94	3.13	6.25	4.23
P	1.52	2.80	2.37	1.61	2.26	2.46
Sa	9.55	3.37	6.22	5.26	6.14	5.83
A	1.54	8.56	5.99	0.00	5.13	6.01
Sp	1.99	2.97	3.42	6.90	3.55	3.19
Total	3.44	3.41	4.21	2.94	4.09	3.67

It is evident from the tables that no consistent correlation has been discovered between the distribution of cases of pellagra and the frequency of use of eggs.

In regard to the relation between use of eggs and the origin of new cases of pellagra, we have data on 861 families, of which 77 developed one or more new cases in 1912 or 1913. The distribution according to frequency of use of eggs is shown in Tables 93 and 94.

TABLE 93.—Distribution of Families According to Frequency of Use of Eggs

Village	Total Families					Families with Incident Pellagra				
	Daily	Habitually	Rarely	Never	Total	Daily	Habitually	Rarely	Never	Total
I	38	43	13	0	94	3	7	2	0	12
W	15	53	24	6	98	2	5	4	0	11
P	37	125	55	9	226	5	9	3	0	17
Sa	27	47	31	3	108	4	2	5	1	12
A	13	34	30	6	83	0	5	4	1	10
Sp	28	90	129	5	252	1	8	5	1	15
Total	158	392	282	29	861	15	36	23	3	77

TABLE 94.—Families with Incident Pellagra in Total Families in Each of the Groups, Per Cent.

Village	Daily	Habitually	Rarely	Never	Rarely or Never	Total
I	7.9	16.3	15.4	....	15.4	12.8
W	13.3	9.4	16.7	0.0	13.3	11.2
P	13.5	7.2	5.5	0.0	4.7	7.5
Sa	14.8	4.3	16.1	33.3	17.6	11.1
A	0.0	14.7	13.3	16.7	13.9	12.0
Sp	3.6	8.9	3.9	20.0	4.5	6.0
Total	9.5	9.2	8.2	10.3	8.4	8.9

There is no evidence of any relationship between the use of eggs by a family and the development of a new case of pellagra in that family.

The 861 families just considered included 4,724 persons free from pellagra at the beginning of 1912. Of these 4,724 persons, 99 contracted the disease during 1912 and 1913. The distribution of these persons according to frequency of use of eggs in their families is shown in Tables 95 and 96.

In these tables it again appears that pellagra originated with almost equal frequency in all the groups, and there is no evidence that its origin was influenced at all by the presence or absence of eggs in the diet. The high incidence, 3.43 per cent., in the 175 persons never using eggs does not appear worthy of much consideration because of the small number of persons concerned and the irregularity of the figures for the different villages.

TABLE 95.—Distribution According to Frequency of Use of Eggs

Village	Total Families					Incident Pellagrins				
	Daily	Habitually	Rarely	Never	Total	Daily	Habitually	Rarely	Never	Total
I	235	256	64	0	555	4	9	4	0	17
W	15	80	140	31	266	2	7	4	0	13
P	210	774	333	61	1,378	5	10	4	0	19
Sa	153	266	202	19	640	5	2	6	1	14
A	64	174	163	36	437	0	7	4	4	15
Sp	149	499	772	28	1,448	1	9	10	1	21
Total	826	2,049	1,674	175	4,724	17	44	32	6	99

TABLE 96.—Incident Pellagrins in Total Population in Each Group, Per Cent.

Village	Daily	Habitually	Rarely	Never	Rarely or Never	Total
I	1.70	3.52	6.25	....	6.25	3.06
W	13.33	8.75	2.86	0.00	2.34	4.89
P	2.38	1.29	1.20	0.00	1.02	1.38
Sa	3.27	0.75	2.97	5.26	3.17	2.19
A	0.00	4.02	2.45	11.11	4.02	3.43
Sp	0.67	1.80	1.30	3.57	1.38	1.45
Total	2.06	2.15	1.91	3.43	2.06	2.10

THE RELATION BETWEEN LOCATION OF A DOMICILE OF AN INCIDENT CASE OF PELLAGRA AND THE DOMICILE OF AN ANTECEDENT CASE

The data accumulated by the house-to-house census in the various mill villages, and our records of the cases of pellagra there, permit us to examine the geographical relationship of the houses in which pellagra has developed to houses in which a case of the disease already existed. We have chosen to divide the non-pellagrin population of

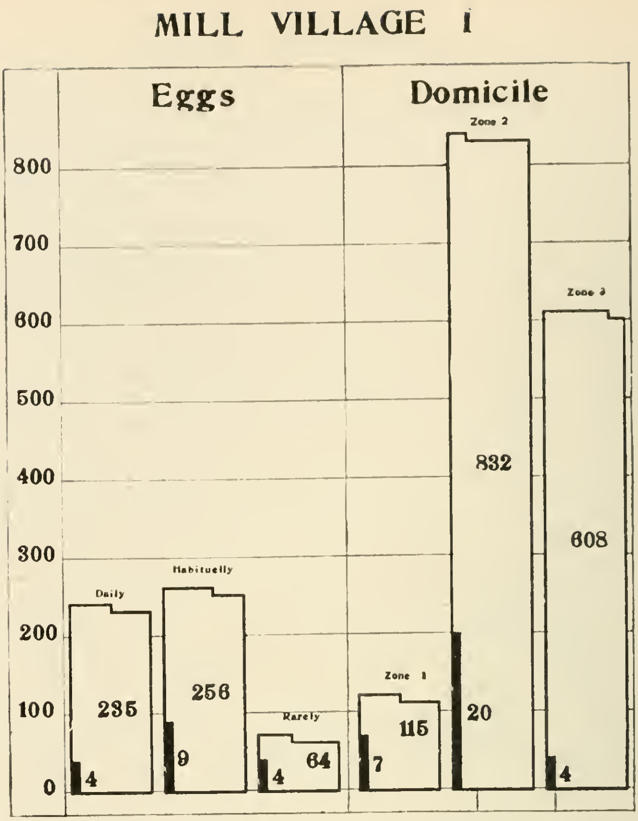


Fig. 21.—The area of each of the three white columns at the left indicates the number of non-pellagrin individuals using eggs daily, *habitually* and *rarely*. The three columns at the right indicate in the same way the number of non-pellagrin individuals living in each of the three domiciliary zones. The included black column indicates in each instance the portion of the respective group which contracted pellagra in 1912 or 1913.

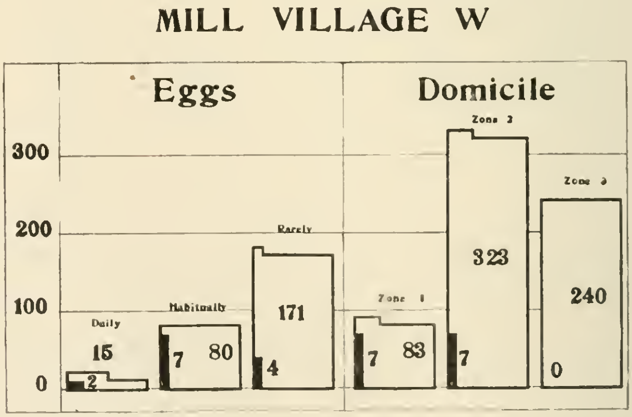


Fig. 22.—The area of each of the three white columns at the left indicates the number of non-pellagrin individuals using eggs daily, *habitually* and *rarely*. The three columns at the right indicate in the same way the number of non-pellagrin individuals living in each of the three domiciliary zones. The included black column indicates in each instance the portion of the respective group which contracted pellagra in 1912 or 1913.

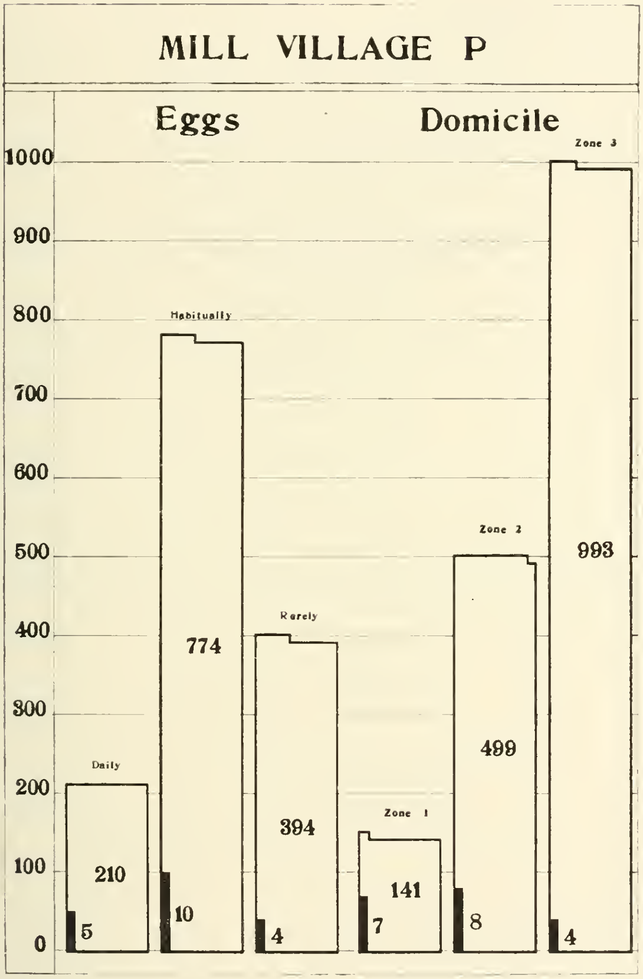


Fig. 23.—The area of each of the three white columns at the left indicates the number of non-pellagrin individuals using eggs daily, *habitually* and *rarely*. The three columns at the right indicate in the same way the number of non-pellagrin individuals living in each of the three domiciliary zones. The included black column indicates in each instance the portion of the respective group which contracted pellagra in 1912 or 1913.

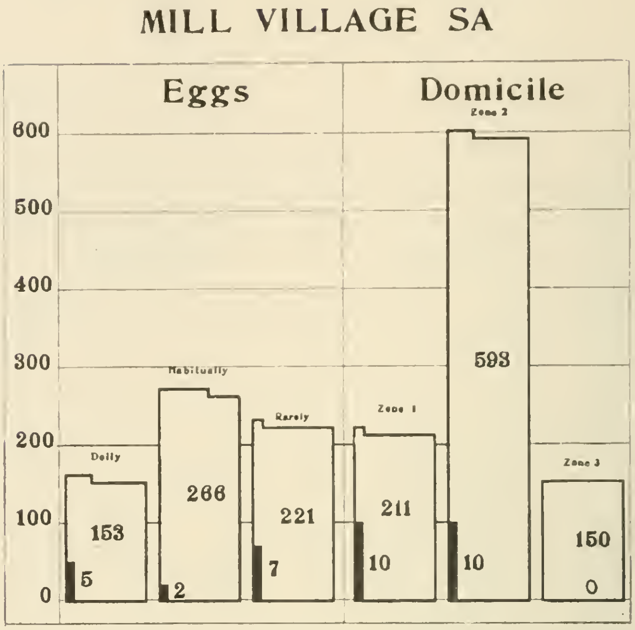


Fig. 24.—The area of each of the three white columns at the left indicates the number of non-pellagrin individuals using eggs daily, *habitually* and *rarely*. The three columns at the right indicate in the same way the number of non-pellagrin individuals living in each of the three domiciliary zones. The included black column indicates in each instance the portion of the respective group which contracted pellagra in 1912 or 1913.

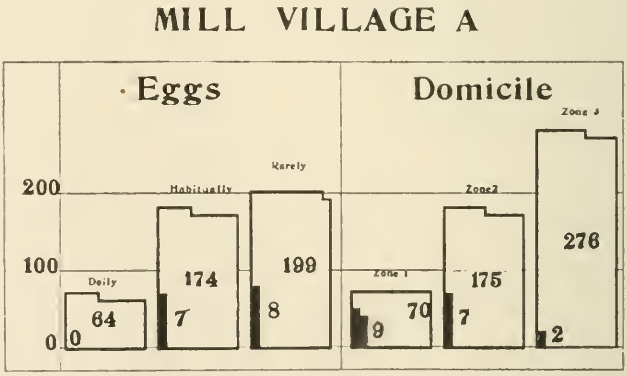


Fig. 25.—The area of each of the three white columns at the left indicates the number of non-pellagrin individuals using eggs daily, *habitually* and *rarely*. The three columns at the right indicate in the same way the number of non-pellagrin individuals living in each of the three domiciliary zones. The included black column indicates in each instance the portion of the respective group which contracted pellagra in 1912 or 1913.

each village into three parts or zones in respect to domicile: first zone, those persons free from pellagra living in the same building in which a patient with pellagra resided during 1912 or the first part of 1913; second zone, those persons, not included in the first zone, who resided in a house adjacent to the house of a pellagrin; third zone, those persons living in houses at distances greater than next door to the domicile of a pellagrin. The second zone included the houses at either side, directly in front, across the street, and directly behind, provided the intervening space was not greater than that of the ordinary mill-village lot (about 100 feet) and also the four houses adjacent on diagonals from the original house. The scheme of the study may be quickly grasped by reference to Figure 27. Each house containing a patient with pellagra during 1912 or early in 1913, whether the

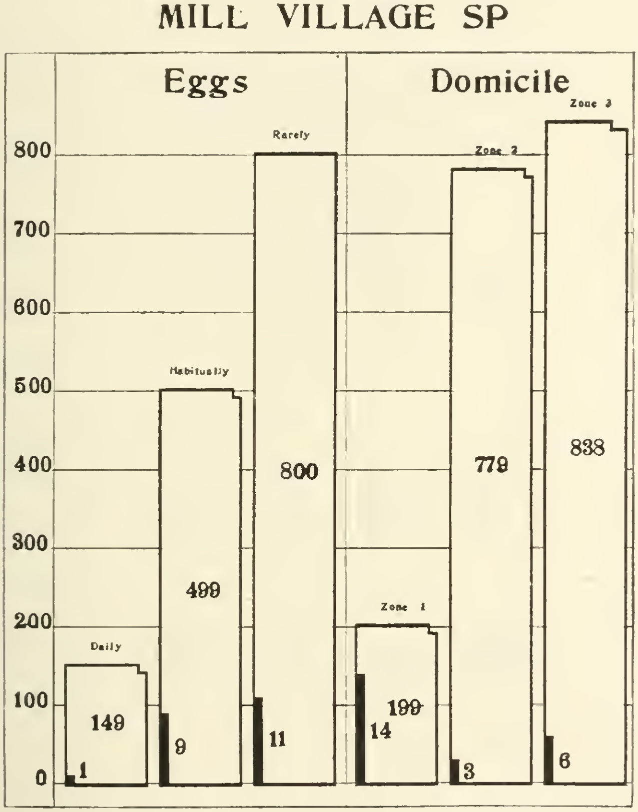


Fig. 26.—The area of each of the three white columns at the left indicates the number of non-pellagrin individuals using eggs daily, *habitually* and *rarely*. The three columns at the right indicate in the same way the number of non-pellagrin individuals living in each of the three domiciliary zones. The included black column indicates in each instance the portion of the respective group which contracted pellagra in 1912 or 1913.

patient moved in there or the case originated there, was considered in turn as the center of such a diagram, and the total persons free from pellagra at the beginning of exposure ascertained for each case as far as our records show them. By adding together the totals for each zone we were then able to ascertain the total number of non-pellagrin population living in the same house with a pellagrin and the total number of cases of pellagra known to develop in this population during 1912 and 1913; in the same way the total number of instances in which a person free from the disease had been domiciled next door to a case of pellagra and total number of instances in which pellagra had arisen under these circumstances; and in the same way for the third zone, we were able to ascertain the number of persons living at a greater distance than next door from a case of pellagra and the total instances in which pellagra had developed during 1912 and 1913 in this population.

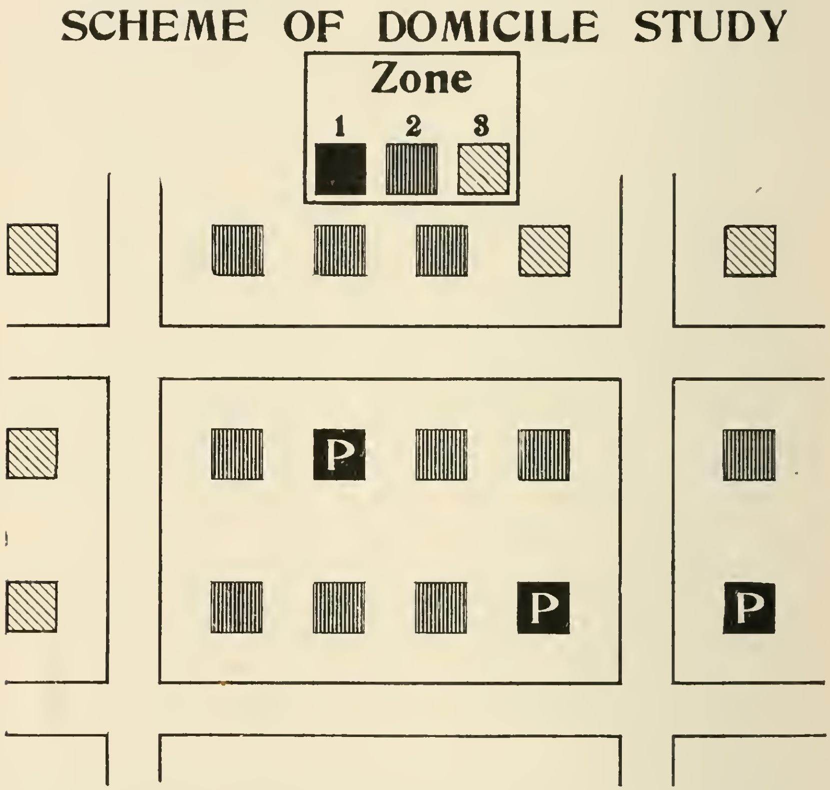


Fig. 27.—The black squares with white letters indicate houses in which cases of pellagra already existed. These houses belong to Zone 1. Houses situated next door belong to Zone 2. All houses situated farther away belong to Zone 3.

Certain difficulties presented themselves from time to time in making this statistical study. It was essential that our knowledge of the cases and of the population employed should be very complete. We therefore limited the antecedent cases to be used to known pellagrins and we excluded from this study those pellagrins who had shown no symptoms of the disease for two years. It was also decided to omit from consideration mere visitors, provided the duration of the visit were less than two weeks. Furthermore, we did not consider as antecedent cases those pellagrins who had moved into the village or had developed symptoms of the disease for the first time within a period of three months preceding the time of our census and search for cases.

Certain arbitrary rules had to be adopted also in regard to the exposed population. Zone 1 included all non-pellagrins known to have lived in the same house with an antecedent case, as defined above, for a period of at least two weeks during 1912 or 1913. Cases of pellagra developing in this zone were considered as incident cases possibly secondary to the antecedent case only when the disease developed after an interval of at least two weeks from the beginning of the domiciliary relationship and within three months in summer or six months in the colder season after the termination of such relationship. Zone 2 included the non-pellagrins living in a house next door to an antecedent case. Those persons who lived next door to several houses in which pellagra existed were considered in relation to each of these houses in turn and added to the population of Zone 2 each time. The population of Zone 2 does not, therefore, mean the number of different persons living next door to a pellagrin, but it designates the number of instances in which a non-pellagrin lived next door to a house containing a pellagrin for a period of at least two weeks during the years selected. Incident cases of pellagra developing in Zone 2 were regarded as possibly secondary to the antecedent case only when symptoms of the disease developed not earlier than two weeks from the beginning of the supposed exposure and not later than from three to six months after its termination. Cases developing in Zone 2 next door to two or more houses containing antecedent cases were credited as secondary to each such case, so that the incident cases in Zone 2 represent not the actual number of persons developing the disease in this zone but the number of instances in which this relationship was followed by the development of incident pellagra. As explained above, the persons who did not contract the disease were treated in a similar way in compiling the statistics. Finally, Zone 3 included all the population living for a period of at least three months in spring and summer or six months in the colder season in houses farther removed than next door from any known case of pellagra;

and any case of pellagra developing in such persons, or in any one not falling within Zone 1 or Zone 2, according to our arbitrary rules, was credited to Zone 3. New cases of pellagra definitely recorded as such, concerning which our information was too incomplete to warrant a final placing in one of these zones, have been temporarily placed in Zone 3. Obviously, it would sometimes happen that a person would first be in Zone 3 and later come to be included in one of the other zones because of change of residence on his part or because a pellagrin would appear next door to him or in his own household. It seemed necessary to adopt such arbitrary rules and to adhere to them throughout this statistical study. Our purpose, of course, was to ascertain whether the proximity of residence to cases of pellagra would show any correlation to the incidence of the disease in these mill villages.

TABLE 97.—Domicile Relationship, Village I, Zone 1

Antecedent Case Present Between October, 1911, and June, 1913			Other Members of the Household			
Case	House	Residence Period	Adults	Children	Total	Incident Pellagrins
514	39	Dec., 1911-May, 1912	17	5	22	1
114	41	Apr., 1911-July, 1913	4	1	5	0
145	44	Jan., 1912-July, 1913	3	3	6	2
148 149	40	1910-July, 1913	3	3	6	1
337	617	Apr. '12-Mar. 31, '13	2	3	5	0
592	617	Mar. 31, '13-July, '13	2	3	5	0
280	611	Feb., 1913-July, 1913	1	1	2	0
330	66	Jan., 1911-July, 1913 Pellagra developed July, 1912	3	4	7	0
164	59	Mar., 1910-July, 1913	1	4	5	0
153 154	54	Nov., 1911-July, 1913	2	2	4	1
150	48	July, 1912-Sept., 1912	12	2	14	1
Total for this portion of the table.....			50	31	81	6

The method of procedure in this study may be illustrated by a sample portion of the original tabulations, from which the data concerning exact location of each house have been purposely omitted (Tables 97, 98 and 99). These fragments of our detailed tables indicate the method employed in compiling the data. It is impossible to print here the complete tabulations because of their extent. The summary of these data is shown in Table 100.

TABLE 98.—Domicile Relationship, Village I, Zone 2

Antecedent Case Present Between October, 1911, and June, 1913			Population Resident in Adjacent Houses				
Case	House	Residence Period	Residence Period	Adults	Children	Total	Incident Pellagrins
514	39	Dec., 1911-May, 1912	Dec., 1911-July, 1913	2	1	3	0
			May, 1912-Sept. 10, 1912	2	1	3	0
			May, 1911-July, 1913	3	2	5	0
			Feb., 1909-July, 1913	2	3	5	0
			Total	9	7	16	0
114	41	Apr., 1911 - July, 1913	Apr. 1908-July, 1913	2	1	3	0
			Feb., 1913-July, 1913	3	0	3	0
			Antecedent Pellagra in Household	..	..	..	..
			Nov., '11 - July, 1913	2	4	6	0
			Jan., 1910-July, 1913	3	1	4	0
			Feb., 1909-July, 1913	2	3	5	0
			Oct., 1912-July, 1913	5	3	8	0
			Total	17	12	29	0
145	44	Jan., 1912-July, 1913	Mar., '12 - Feb., 1913	4	4	8	0
			Jan. 1911-July, 1913	3	5	8	2
			Mar., '13-July, 1913	4	0	4	0
			Feb., 1913-July, 1913	3	0	3	0
			Dec., 1912-July, 1913	3	4	7	0
			Aug., '12-Mar., 1913	5	5	10	0
			June, '13,-July, 1913	2	0	2	0
			Antecedent Pellagra in Household	..	..	..	..
			Apr., 1913-July, 1913	2	2	4	0
			Mar., '13-July, 1913	2	0	2	0
			Apr., 1913-July, 1913	2	1	3	0
			Dec., '11-Mar., 1913	3	0	3	0
			July, '12-Mar., 1913	5	4	9	0
			Total	38	25	63	2
148 149	40	1910-July, 1913	*Antecedent Pellagra in Household*	..	..	..	..
			Jan., 1911-July, 1913	4	4	8	1
			Dec., 1912-July, 1913	2	5	7	1
			Mar., '13-July, 1913	4	0	4	0
			Feb., 1913-July, 1913	3	0	3	0
			Apr., 1913-July, 1913	2	2	4	0
			Mar., '13-July, 1913	2	0	2	0
			Apr., 1913-July, 1913	2	1	3	0
			Mar., '13-Apr., 1913	2	2	4	1
			1910-Feb., 1913	5	4	9	0
			Total	26	18	44	3
Total for this portion of the tab			....	90	62	152	5

The result of this compilation of data in regard to location of domicile is very striking. Of the 819 non-pellagrin individuals who lived in the house in which pellagra existed at the time, 54 acquired the disease during 1912 and 1913. Of the 3,201 instances in which non-pellagrin individuals lived next door to a house in which pellagra was present, the disease developed in this exposed population in 55 instances during 1912 and 1913. Of the 3,105 persons who lived in houses farther away than next door to a pellagrin, 16 contracted the disease during the two years. The respective percentages are 6.59 for Zone 1, 1.72 for Zone 2 and 0.52 for Zone 3. As far as these mill villages are concerned it is clearly evident that a high degree of correlation existed between proximity of domicile to an existing case of pellagra and the origin of new cases in the population of such a domicile. In other words, the new cases of the disease developed almost exclusively in small foci, within which one or more old cases of the disease already existed.

TABLE 99.—Domicile Relationship, Village 1, Zone 3

Residence Period	Adults	Children	Total	Incident Pellagrins	Excluded from Zone 3 by Presence of Pellagra in Same or Adjacent House
Jan., 1913 - July, 1913	12	1	13	0	1911 to Aug., 1912.
May, 1911-July, 1913	3	2	5	0	1911 to Aug., 1912.
April, 1913-July, 1913	4	2	6	0	July, 12, '12, to Sept., 1912.
Nov., 1911-July, 1913	5	0	5	0	July, 12, '12, to Sept., 1912.
Oct., 1910 - July, 1913	2	1	3	0	July, 12, '12, to Sept., 1912.
Dec., 1911-July, 1913	2	1	3	0	1911 to March, 1912.
Feb., 1913 - July, 1913	2	2	4	0	1911 to March, 1912.
1909-July, 1913	2	3	5	0	1911 to March, 1912; July, 1912, to July, 1913.
Nov., 1911-Dec., 1912	2	1	3	0	....
Jan., 1913 - July, 1913	2	4	6	0	....
Jan., 1913 - May, 1913	2	1	3	0	....
May, 1913 - July, 1913	2	1	3	0	....
Apr., 1908 - July, 1913	2	0	2	0	June, 1912, to July, 1913.
Nov., 1909-July, 1913	3	4	7	2	July, 1912, to July, 1913.
Feb., 1910-July, 1913	4	0	4	0	1911 to March, 1912; June, 1912, to July, 1913.
Apr., 1908-July, 1913	2	1	3	0	1911 to March, 1912; June, 1912, to July, 1913.
Apr., 1911-July, 1913	5	0	5	0	June, 1912, to July, 1913.
Jan., 1912 - Jan. 1913	3	0	3	0	July, 1912, to July, 1913.
Nov., 1911-July, 1913	2	4	6	0	....
Nov., 1911-Sept., 1912	2	4	6	0	....
Apr., 1913 - July, 1913	2	1	3	0	....
Total for this portion of the table	65	33	98	2	

The figures presented above have been subjected to a critical scrutiny with especial attention to the accuracy and the completeness

TABLE 100.—Incidence of Pellagra in the Population of Different Zones According to Location of Domicile in Relation to Domicile of an Antecedent Pellagrin

Village	Zone 1			Zone 2			Zone 3		
	Instances of Exposure	Incident Pellagrins	Incidence Per Cent.	Instances of Exposure	Incident Pellagrins	Incidence Per Cent.	Instances of Exposure	Incident Pellagrins	Incidence Per Cent.
I	115	7	6.09	832	20	2.40	608	4	0.66
W	83	7	8.43	323	7	2.17	240	0	0.00
P	141	7	4.96	499	8	1.60	993	4	0.40
Sa	211	10	4.74	593	10	1.69	150	0	0.00
A	70	9	12.86	175	7	4.00	276	2	0.72
Sp	199	14	7.04	779	3	0.38	838	6	0.72
Total	819	54	6.59	3,201	55	1.72	3,105	16	0.52

of our records and to the possibility of association with other cases of pellagra besides the antecedent cases here considered. In regard to Zone 1, this scrutiny has shown a high degree of accuracy and completeness. In many instances the household in which an old case of pellagra existed had been examined and detailed record of it made previous to the development of the secondary cases. The development of new cases presented opportunity to verify and extend the records already made. In Zone 2, the cases have not, on the whole, been so completely studied and in some of these the location of previous residences is incompletely known and, in some instances, the possibility of close association with other pellagrins by visits is clearly indicated by the records. In Zone 3 there are only 16 incident cases and they will be considered briefly in order.

Patients 152 and 157, at village I, were father and son. They had been living in the same house for three years, during which time no patient with pellagra had resided in the immediate vicinity. The nearest case was at a distance of five houses, in the village. Both of these persons developed pellagra about the same time, in June, 1912. The father of the older patient, grandfather of the boy, is said to have pellagra. He lives at a distance of about a mile in the country. We have no further information in regard to him. The wife and mother in this family has been in poor health for some years, but there is no definite history and no evidence of pellagra in her. The two cases have been placed in Zone 3. As far as relation of domicile is concerned, one cannot be considered secondary to the other. A third case in village I is Patient 153, a little girl aged 5. Her family had lived in the village part of the time ever since her birth. During the spring and summer of 1911 they lived on a farm some distance from the village and moved to the present residence the last of November, 1911. The little girl suffered from diarrhea since early in the spring of 1912 and developed the erythema in March, 1912. We have not been able to obtain information concerning the associations at the farm. The case therefore falls in Zone 3. The fourth case in village I is Case 280, a woman aged 28, living in a house removed from cases of pellagra by one intervening house. Her father-in-law, Patient 316, died of pellagra in August, 1911, in the house next door but one from the house in which this patient was living. In Case 280, erythema developed for the first time in July, 1912. Evidence of living next door to a pellagrin is absent in this case and it is therefore placed in Zone 3.

In village W there are no cases in the third zone to be considered.

In village P there are four cases in Zone 3. Case 54 was in a boy aged 12, who developed pellagra for the first time in May, 1912.

He had lived in the same house in this village since March 6, 1912, having come from Lee County, Va. There was no recognized case of pellagra among the persons living in any of the adjacent houses at this time. In a house next door but one, there was a little girl who had suffered from pellagra for about a year. In the spring of 1912 three cases of pellagra in children originated in this immediate vicinity; one in the younger sister of this original case, one in a girl aged 10, who lived next door, and one in this boy we are now considering, Case 54. The three families lived in adjacent houses, the house in which the 10-year-old girl lived being between the house of the original case and this Case 54. These three children all developed pellagra about the same time and it is therefore impossible to regard the case of this boy as secondary to that in the girl living next door to him. He has been placed in the third zone. In Case 630, a girl aged 8, pellagra developed for the first time in July, 1912, while the patient was living in a house in Zone 3. Her mother, Patient 299, died of pellagra in June, 1911. The child lived with her mother in the same house in which the child developed the disease later. Her mother, however, was taken from this house shortly before she died, to her father's house, grandfather of Patient 630, a short distance away in the same village, where she died. The grandfather developed pellagra during 1911. The evidence of close association between the child, its mother and grandfather, is, of course, very clear; but inasmuch as the domicile in which the erythema developed is further away than next door from the grandfather's house and the mother was removed from this domicile about a year before the child developed the disease, the case is placed in Zone 3. Patient 640 was a woman aged 27 who had been living in the same house since 1910. She developed pellagra for the first time in June, 1913. The house is next door but one to a house containing two cases of the disease which developed in 1912. In the same house where this woman lived there was a boy, Patient 521, who had pellagra in the summer of 1911. He had lived in this house since 1908 and moved away in September, 1911. Patient 557 was, of course, associated with this boy in the same house during 1911, but inasmuch as the record shows that she developed her erythema in June, 1913, nearly two years after the other patient had left, she is placed in Zone 3. Patient 634 was a woman aged 35. She developed erythema for the first time the latter part of July, 1913. She had been living in the same house since February, 1912, and there was no case of pellagra within a radius of four houses. Previous to February, 1912, she had lived on the other side of the river near her father's house. The exact location of her residence there has not been ascertained. Her father, Patient 300, developed pellagra in 1911 and died of pellagra in August, 1912. The evidence of association between these two cases has not been definitely ascertained, but even if it were fully known, the domicile in Case 634 would, nevertheless, remain in Zone 3. This woman died of pellagra, Sept. 23, 1913.

In village Sa we find no cases of pellagra which developed in houses at a greater distance than next door from a previous case.

In village A, two persons developed the erythema for the first time while living in houses which did not contain a case of pellagra and which were not adjacent to such houses. One of these, Patient 594, was a woman aged 30, who had lived in her present residence since October, 1912. She developed erythema for the first time in June, 1913. Her residence previous to October, 1912, had not been definitely ascertained. During the winter of 1912 and 1913, that is, from November, 1912, to January, 1913, Patient 570 lived in the same house. Patient 570 is believed to have developed erythema for the first time in June, 1913, after leaving this house, but her sister states that she had suffered from pellagra also during the summer of 1912. The exact information in regard to this point is lacking. As we do not know with certainty of any association in the household or of any next-door relationship to a previous case, we have placed Case 594 in the third zone.

Case 579 was in a boy aged 4. He had been living in his present residence since December, 1912, and developed erythema for the first time in June, 1913. During this interval, from December to June, there was no recognized case in the same house or in any of the adjacent houses, excepting one. In this one exceptional house, three cases of pellagra developed in 1913, one on May 1, one on June 19 and one on June 25. Two of these cases were in young children. In our study of relation of domicile we decided not to consider a case as a center of origin unless the patient had resided in this particular village previous to April, 1913. It is therefore impossible to include this case, 579, in our Zone 2. This child, however, played with the other children a large part of the time, and there is no question that association with other pellagrin children in the immediate neighborhood was very close.

In the village Sp there are six cases to consider. Patient 747 was a woman aged 44 who had been living in her present residence since 1907. She was observed to have pellagrous erythema in October, 1913. She refused to give us information concerning her history, except to say that she had been ailing for three years. There was no antecedent case of pellagra within a radius of four houses and, in the absence of more complete information, this case has been credited to Zone 3. Case 748 was in a woman aged 44, who was living directly across the street from Case 747. She had been in this house since 1906. Erythema developed for the first time in August, 1913. This patient was also very reluctant in giving her history. It is perhaps justifiable to consider her as a case developed next door to Case 747, but in the absence of more complete information we have placed her in Zone 3. Patient 548, a girl aged 5, had been living in her present residence since September, 1913. The nearest antecedent case of pellagra was three doors away. The little girl developed erythema for the first time about June 1, 1913. Her parents both worked in the mill, and she played with the neighbors' children most of the time. We were unable to find that she actually lived in the house with a case of pellagra or in a house next door to such a case. Patient 718, a woman aged 42, had been living in her present residence since April, 1912, and she developed erythema for the first time in May, 1913. Her previous residence had been across the street from a case of pellagra. Her residence in 1913 was an apartment house in which there were no other cases of the disease. The association in the previous house was too remote to come within the limits which we have set for ourselves in this study. She has been placed, therefore, in Zone 3.

This brief examination of the cases originating in a house farther away than next door from an antecedent case of pellagra shows that in most of the instances there is evidence of previous close association with cases of pellagra or that our information is too incomplete to warrant a final opinion in regard to this question. It serves to strengthen rather than weaken the indication shown by the tabulated figures. We are forced to conclude that the origin of pellagra in persons living at a distance from previous cases of the disease in these particular mill villages was relatively very uncommon in 1912 and 1913.

DISCUSSION OF FOODS AND OF LOCATION OF DOMICILE AS ETIOLOGICAL FACTORS IN PELLAGRA

The preceding pages have presented the data concerning the use of particular foods and concerning the location of domicile in relation to pellagra, obtained by an intensive study of the inhabitants of six mill villages. It remains to discuss these facts presented and their bearing on the problem of the causation of pellagra.

In the first place the data do not support the theory that pellagra is an intoxication, the recurrent manifestations of which are due to the continued use of maize. The simple fact is that the portion of the population rarely or never using shipped corn contained a relative excess of pellagrins as compared with those using this food daily. Of those persons using shipped corn daily, 3.02 per cent. were pellagrins; of those using it habitually, 3.92 per cent., of those using it rarely or never, 5.49 per cent. The relation to local corn-meal was somewhat similar. Of those persons using it daily, 2.99 per cent. were pellagrins; of those using it habitually, 3.90 per cent.; of those using it rarely or never, 3.83 per cent. The study of corn-meal, both kinds considered together, gave an even more emphatic result. Of those persons using it daily, 3.13 per cent. were pellagrins; of those using it habitually, 4.30, and of those using it rarely or never, 6.02 per cent. were pellagrins. It would seem justifiable to dismiss at once the idea that continued use of maize was the important factor in producing the symptoms of pellagra in the population of these villages.

A precisely similar conclusion may be drawn concerning the supposed toxic influence of canned foods as a primary factor in the causation of the symptoms of pellagra. Of the 89 persons using canned foods daily, none were pellagrins; of those using these foods habitually, 3.25 per cent. were pellagrins; of those using them rarely or never, 4.12 per cent. were pellagrins.

So far as we are aware, no one has yet ascribed the causation of pellagra to the use of fresh meat, milk or eggs, and we do not perceive any reason for giving them any consideration as direct causes of this disease.

In the second place it is necessary to examine the food data from the point of view of those who regard deficiency in total amount or in the quantity of particular elements of the food as the real cause of the symptoms and lesions of pellagra. The data presented in the preceding pages have little bearing on the theory that pellagra is the direct result of a general deficiency in diet. Our observation of pellagra in apparently well-nourished growing children, however, as well as in apparently well-nourished adults, have made this conception appear unworthy of consideration. The theory that a deficiency in some special dietary constituent may cause pellagra seems worthy of much more attention, especially because of the brilliant success attained in the prophylaxis of beriberi by measures based on a similar theory of causation in that disease. In general the dietary in these villages showed considerable variety. Most of the families possessed small gardens, and fresh vegetables were everywhere used in season. The data concerning the use of fresh meat, milk and eggs would appear to have a particular bearing on this question, because these foods might be supposed to contain the particular substance or substances, a lack of which would be assumed to cause the signs and symptoms of pellagra. Of the 82 persons in families using fresh meat daily, four were pellagrins, 4.88 per cent.; of the 2,591 persons in families using this food habitually, 3.74 per cent.; of the 2,460 persons in families using this food rarely or never, 3.98 per cent. were pellagrins. Most significant of all, perhaps, was the fact that of the 263 persons never using fresh meat, only 4, or 1.52 per cent., were pellagrins, a figure considerably below the average morbidity for the whole population considered, namely, 3.88 per cent. Evidently the fresh meat used in these villages cannot be regarded as the essential pellagra-preventing food.

The data concerning the use of milk do show evidence of a relation of this food to pellagra. Of the 3,171 persons in families using milk daily, 89, or 2.81 per cent., were pellagrins, while of the 619 persons avoiding the use of milk, 43, or 6.95 per cent. were pellagrins. An analogous apparent effect of milk in preventing the development of new cases of pellagra was also evident in the study of the incident cases. We do not regard deficiency of milk in the diet as the cause of pellagra, however, because pellagra existed in many families in which milk was used daily and we have records of many cases of pellagra in persons who habitually drank sweet milk or buttermilk, and of several who drank buttermilk daily. Furthermore, it is well known that milk and milk products, although valuable dietary elements for adult man, are not absolutely essential for his nutrition. A further reason for refusing to accept deficiency in milk as the essential cause of pellagra is its complete failure to account for the focal distribution of the incident cases so clearly brought out in the domiciliary study. Nevertheless, as far as these data are concerned, milk, including buttermilk, stands out prominently as the one dietary element which seems to have had any influence at all on the prevention of pellagra, and the number of persons included in each of the groups using this food daily, habitually, rarely and never is sufficiently large to give some weight to the evidence.

The use of eggs exerted no apparent influence on the existence of pellagra and only an uncertain influence on the origin of new cases. The pellagra morbidity in the 170 persons avoiding the use of eggs was only 2.94 per cent., while the average for the total population considered in this relation was 3.67. Of the population considered in relation to the incidence of pellagra, 175 persons never used eggs. Of these, 6, or 3.43 per cent., contracted the disease, an incidence somewhat above the average, 2.10 per cent., for the total population considered. The small number of persons in this group (175), and the great irregularity of the figures in the different villages, render the average figure, 3.43 per cent., very unreliable as an indication of the value of eggs in preventing pellagra. We are inclined to ascribe no importance whatever to this article of diet.

The omission or restriction of the use of corn-meal does show a slight associated increase in pellagra. For example, the 123 persons who avoided the use of corn-meal altogether included 6 pellagrins, a morbidity of 4.88 per cent., as against an average of 3.81 per cent. in the total population considered. Similarly, of the population considered in relation to incident cases, 123 persons avoided the use of corn-meal and four of these contracted pellagra, an incidence of 3.25 per cent., as against an incidence of 2.14 per cent. for the whole 5,056 persons considered. We do not believe that omission of maize from the diet has contributed essentially to the increase of pellagra. The real explanation seems to be that some of those persons who already had the disease have restricted their use of maize and in some cases other members of their families have done the same. As has been shown in the domiciliary study, those persons closely associated with preexisting cases of pellagra contracted the disease most frequently. The slightly higher incidence among those taking little or no corn-meal is probably to be explained, therefore, by a relatively closer association of these persons with previous cases of the disease; an association which tended to produce two results, namely, restriction in the use of corn-meal and also a higher incidence of pellagra.

In the third place we wish to consider the bearing of the facts presented on the theory that pellagra is an infectious disease. The relationships discovered in the domiciliary study seem to us to be conclusive evidence of the correctness of this view. It is evident that, so far as was observed in the population of these mill villages in 1912 and 1913, the location of one's domicile in the same house with, or next door to a pellagrin, or farther away, had a greater and more definite relation to the subsequent development of pellagra than did the use or avoidance of any of the foods to which attention has been given. The observations indicate very clearly that pellagra actually spread in these villages from a preexisting case as a center, and that it spread readily only within a very small area surrounding such a preexisting case.

In order to present in a graphic manner the apparent relation of these dietary elements and of location of domicile to the origin of new cases of pellagra we have constructed a series of charts to illustrate the data in regard to incident cases, showing by actual area of each column the number of persons in families using each of the foods daily, habitually and rarely (including never) in each of the mill villages. These charts (Figs. 3 to 26, inclusive) show at a glance the relative number of the population in each of the frequency groups in respect to each of the seven foods studied and, in a precisely similar way, the population in each of the three domiciliary zones in the same village. In each of the large columns a smaller black column at the lower left corner indicates the actual number of that group which contracted pellagra during 1912 and 1913. The variation in the size of the different dietary and domiciliary groups in the different villages is shown very clearly.

Figure 28 shows the incidence of new cases per ten thousand in the total population in the six villages using each of the foods daily, habitually and rarely (including never). The *higher* incidence of pellagra in those who rarely or never used corn-meal, the *higher* incidence in those who did use fresh meat daily, the *absence* of incident cases among those using canned foods daily, and the *lower* incidence among those using milk daily can be appreciated at a glance. It is, of course, necessary to refer to the preceding charts or to the tabulated data to ascertain the size of each group, in order to know the significance of the ratios shown here.

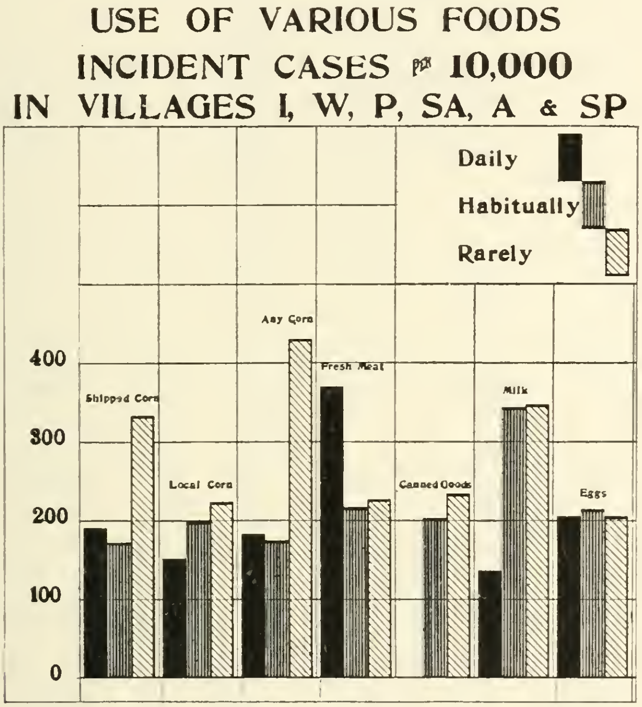


Fig. 28.—The incidence of new cases of pellagra in the total population of the six villages in each of the three groups summarized according to frequency of use of the various foods.

Figure 29 shows in a similar way the incidence rate per ten thousand in each of the three domiciliary zones for each of the six mill villages and, at the right under "Av.," for the total population considered together. This distribution of incident cases can be accounted for, in our opinion, only by the conception that pellagra is an infectious disease spreading from a preexisting case as a center. Further-

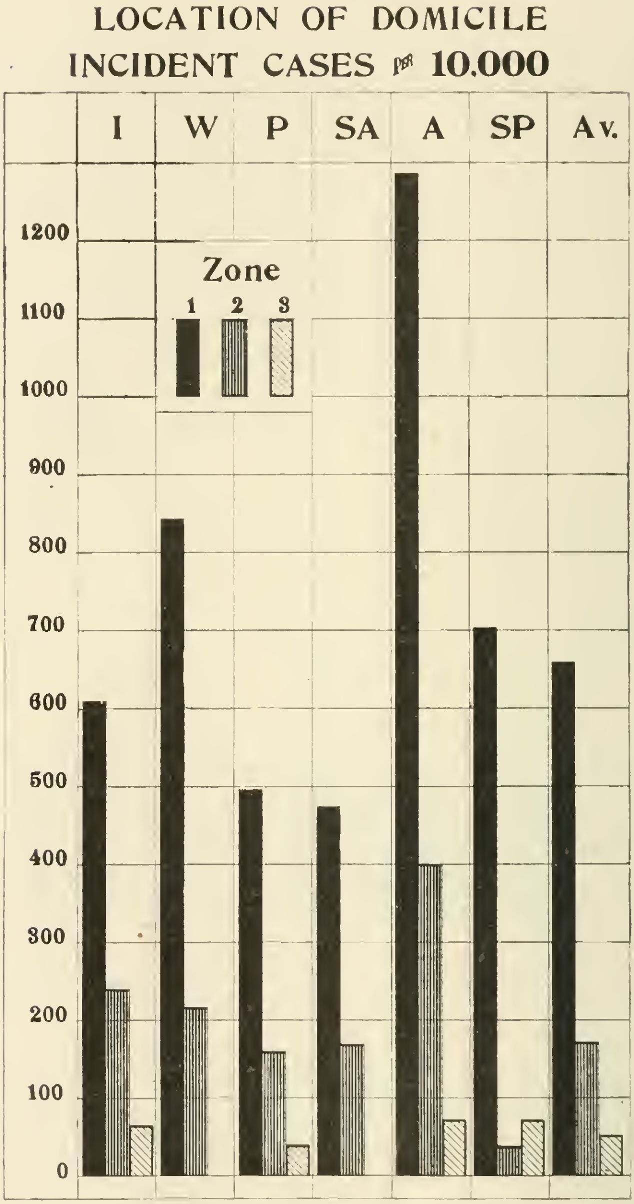


Fig. 29.—The black columns indicate the incidence of new cases of pellagra among persons living in the same house with a preexisting case. The columns striped vertically indicate the incidence in the population living next door. The columns striped obliquely indicate the incidence in the population living within the respective village, but farther away than next door from a preexisting case of pellagra.

more, it seems in general not to be very readily transmitted even to those in the same house, and its spread to those at a greater distance is very much less common. It should be remembered that nearly two years elapsed while the new cases on which this chart is based were originating.

Summary

1. Pellagra spread from a preexisting case as a center in the six villages here studied.

2. It was transmitted to new victims only through very short distances and chiefly to those immediately associated in the home with a preexisting case of the disease.

3. Frequent use of corn-meal as an article of diet was not a factor in the causation of pellagra in these villages.

4. There was discovered no evidence that canned goods have anything to do with the causation of pellagra.

5. Frequent use, even daily use, of fresh meats and of eggs afforded no relative protection from pellagra in these villages.

6. The daily use of milk seemed to diminish to some extent the danger of contracting pellagra in these mill villages in 1912 and 1913, although its use did not fully insure against the development of the disease.

NITROGEN AND SULPHUR METABOLISM IN A CRETIN *

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In spite of a number of observations on the balance of nitrogen, phosphorus, calcium and other elements in the metabolism of cretins, there appears to be only one series of experiments on record in which careful determinations of the amounts of the various nitrogenous constituents of the urine were made. These were reported by Scholz[1] in 1906. According to him, the nitrogen partition is practically normal. Unfortunately, of the methods then available, only those for the determination of nitrogen, urea and ammonia can be considered at all reliable. More recently, McCrudden[2] studied the excretion of creatinin and creatin in a cretin. The amount of creatin eliminated was much less than in other types of arrested development and was greater on a carbohydrate-rich diet than on one rich in fat. The sulphur metabolism of cretins has, apparently, never been studied.

Nitrogen and Sulphur Metabolism—

Date Jan., 1913	Nitrogen in Food, gm.	Nitrogen Excretion in Gm.					
		Total	Urea	Ammonia	Creatinin	Creatin	Uric Acid
8	5.38	3.998	3.053	0.163	0.0550	0.0115	......
9	5.38	2.440	2.300		0.0339	0.0137	......
11-13	4.27*	3.000	2.520	0.248	0.0470	0.0120	0.0408
19-20	5.22*	3.150	2.394	0.347	0.0450	0.0260	0.4000
21-22	5.60*	3.634	3.144	0.287	0.0579	0.0185	0.0438

* In the last three periods the figures are for twenty-four hours.

An opportunity to study the metabolism in a cretin girl presented itself and it was considered advisable to pay particular attention to the distribution of nitrogen and sulphur in the urine.

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* Submitted for publication Nov. 17, 1913.

* From the Chemical Laboratory of the Montefiore Home.

[1] Scholz: Ztschr. f. exper. Path. u. Therap., 1906, ii, 271.

Examination of the results show few deviations from the normal. The absorption of nitrogen is poor. The ratio of ammonia-nitrogen to total nitrogen is rather high. The creatinin output is very low, much less than in McCrudden's[9] case and a considerably greater amount of creatin is excreted. Aside from these the findings seem to be quite normal.

The patient, A. S., aged 7, weighed 15.5 kg. (34 pounds). Her general behavior was that of a sluggish child of 8 or 9 months. The thyroid was not palpable, and in every way she presented the picture of a typical cretin.

The girl was placed on a diet consisting of farina, oatmeal, milk, toast, butter and eggs, and an attempt was made to collect the urine and feces separately. Various devices were used, but none were satisfactory. It was noted, however, that defecation occurred only once in three or four days. This made the collection of urine free from feces rather easy. A bed such as that described by Du Bois[2] was used. A trough of galvanized iron was arranged so as to collect all the urine and conduct it into a bottle containing thymol.

—in Case of A. S., a Cretin

Sulphur Excretion in Gm.			Percentage of Total Nitrogen as					Percentage of Total Sulphur as	
Total	Total SO_4	Inorg. SO_4	Urea	Ammonia	Creatinin	Creatin	Uric Acid	Sulphate	Inorganic Sulphate
.....	.....	.....	89.87	4.78	1.62	0.34	....	....	....
0.205	0.192	0.184	94.26		1.39	0.56	....	93.5	89.8
0.259	0.240	0.231	84.05	8.27	1.56	0.42	1.36	92.6	89.0
0.273	0.253	0.245	76.09	11.01	1.42	0.92	1.26	92.8	90.0
0.290	0.256	0.241	86.49	7.91	1.59	0.51	1.21	88.5	75.9

This trough was washed with distilled water every day and the washings were added to the urine. If defecation occurred, the urine was not used. When the urines of two or more days were combined, the earlier specimens were kept in a refrigerator until the later urines were obtained. The following methods were used: Total nitrogen, Kjehldahl-Gunning; urea, Benedict;[3] ammonia, Folin;[4] creatinin and creatin, Folin;[5] uric acid, Folin-Shaffer;[6] total sulphur, Benedict;[7] inorganic and total sulphate, Folin.[8] The nitrogen of the food was calculated according to the data given in Bull. 28, U. S. Dept. Agric.

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[2] Du Bois: Am. Jour. Dis. Chil., 1911, ii, 419.

[3] Benedict: Jour. Biol. Chem., 1910, viii, 405.

[4] Folin: Ztschr. f. physiol. Chem., 1902, xxxvii, 161.

[5] Folin: Ztschr. f. physiol. Chem., 1904, xli, 222.

[6] Folin and Shaffer: Ztschr. f. physiol. Chem., 1900, xxxii, 552.

[7] Benedict: Jour. Biol. Chem., 1909, vi, 363.

[8] Folin: Jour. Biol. Chem., 1906, i, 131.

LYMPHOCYTE INCREASE AND ALTITUDE *

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AND
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During the past few years much accurate and careful work has been done to establish the exact influence of altitude on the blood. The accumulated evidence would seem to have settled the fact that there is a definite increase in the number of erythrocytes and in the content of hemoglobin. At a moderate altitude of about 6,000 feet this increase is at least 10 per cent. in both instances. No increase in the total number of leukocytes has been found. Little attention was paid to the differential blood-count at various altitudes until recent years.

In April 1909, Webb and Williams[1] first called attention to an absolute increase in the lymphocyte or mononuclear element of the blood as an effect of altitude. In this and the previous year evidence regarding the defensive behavior of the lymphocytes in tuberculosis had been brought forward, notably by Bartel,[2] Bergel[3] and Marie and Fiessinger.[4] The latter demonstrated that lymphocytes contain a lipolytic ferment which can destroy the waxy coat of the tubercle bacillus. These connected observations suggest a partial explanation of the apparently low incidence of tuberculosis among the inhabitants of high altitudes and also of the reputed curative virtues for the tuberculous of mountain resorts.

Stäubli,[5] in 1910, published a few figures which showed an increase in the lymphocytes at a high altitude. Quite recently Baer and Engelsman[6] of Davos, have thoroughly corroborated this observation.

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* Submitted for publication, March 14, 1914.

* From the Laboratory of Cragmor Sanatorium, Colorado Springs, Colo.

[1] Webb and Williams: Some Immunity Problems in Tuberculosis, Colorado Med., April, 1909; Some Hematological Studies in Tuberculosis, Tr. Nat. Assn. for the Study and Prevention of Tuberculosis, 1909.

[2] Bartel: Experiments in Immunization Against Tuberculosis, International Congress on Tuberculosis, 1908.

[3] Bergel: Fat-Splitting Ferment in Lymphocytes, München. med. Wchnschr., 1909, No. 2, p. 64.

[4] Marie and Fiessinger: Compt. rend. Soc. de biol., July 23, 1909.

[5] Stäubli: Beiträge zur Kenntnis des Einflusses des Hochgebirgsklimas, Verhandl. d. deutsch. Cong. f. inn. Med., 1910.

[6] Baer and Engelsmann: Das Leukocytenbild bei Gesunden und Lungentuberkulosen in Hochgebirge, Deutsch. Archiv. f. klin. Med., 1913, cxii.

O. M. Gilbert[7] also confirmed the work of Webb and Williams by finding greater percentages of lymphocytes in specimens of blood taken at an altitude than in specimens taken at sea-level, although below sea-level he observed even higher counts. Too few and too incomplete observations, however, were made to be of marked significance.

During the year 1911, when discussing the present attitude toward climate, Dr. E. R. Baldwin[8] of Saranac Lake, wrote:

> If Dr. Webb's observations about the effect of altitude should be confirmed, I think it would be an important element in the matter of the treatment of tuberculosis. So far I have not heard of any confirmation nor any control experiments on a large scale which would make assertions very safe yet. I hope that he and his associates will continue to study it and give us some facts about it.

Realizing the importance of these remarks, we have continued to study at every opportunity the influence of altitude on the differential blood-picture and slowly and carefully to collect scientific data on this question.

During the summer of 1911 blood-films were obtained from the members of the Anglo-American Physiological Expedition[9] to Pike's Peak during their six weeks' residence at an altitude of 14,000 feet. These specimens exhibited a very marked increase in the percentage of lymphocytes.

This paper will report a series of differential leukocyte counts on 100 male students at Cornell Medical College in New York City, and on 100 male students at Colorado College in Colorado Springs. Each student was ascertained to be in normal health and between the age of 20 and 30. All counts were taken at twelve noon before lunch and when no strenuous exercise had been taken during the morning. The technic used in New York was identical with that used in Colorado, every detail having been previously arranged. The first blood was wiped away and the specimen was taken from a free flowing drop either from finger or ear. As thin a film as possible was obtained on a pair of cover-slips. Hastings' and Wright's stains were used interchangeably. In every instance at least 200 cells were counted and more often 300. A total leukocyte count was made at the same time.

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[7] Gilbert, O. M.: Differential Leukocytes at Various Altitudes, Colorado Med., December, 1911.

[8] Baldwin, E. R.: Quoted by A. M. Forster. The Present Attitude Toward Climate, Tr. Seventh Annual Meeting Nat. Assn. for the Study and Prevention of Tuberculosis, p. 230.

[9] Webb: Phil. Tr. Roy. Soc. London, Series B, Vol. ccv, p. 312. Studies in Tuberculosis, Johns Hopkins Hosp. Bull., August, 1912.

In 156 cases a red count was also made as indicated. Nearly all the films taken in New York were recounted in this laboratory. The counting extended over a period of several months during the year 1913.

Table 1 presents a summary of the results of this work. It will be noted that we group all the basophilic mononuclear cells together and make no attempt to classify them according to origin, function or fate. Whether the transitional cells should be grouped with the large mononuclear cells is, of course, a much-disputed question.

TABLE 1.—Differential Leukocyte Counts on One Hundred New York Students and One Hundred Colorado Students

	New York Students*	Colorado Students	Percentage Increase
Mononuclear Percentages—			
Average	*34.4 (35.3)	42.8	24 (21)
Mean	34.0 (34.0)	42.5	
Extremes	27.0-45	26.5-60	
Total Mononuclears per cm.—			
Average	2,522	3,279	30
Mean	2,450	3,172	
Extremes	1,320-4,300	2,268-5,332	
Polymorphonuclear Percentages—			
*Average	62.3	55.0	
Extremes	52.0-70	35.0-73	
Erythrocytes—			
Average	4,875,920	5,984,000	22
Mean	4,864,000	5,952,000	
Extremes.	4,416,000 to 5,480,000	5,156,000 to 7,216,000	
Total Leukocytes—			
Average	7,340	7,795	
Mean	7,000	7,440	6

* Most of these slides were recounted in Colorado and the average was found to be 35.3 per cent., the mean 34 per cent. This shows a close comparison in the counting in New York and Colorado in regard to the total mononuclear elements.

The blood-counts were made in New York by Dr. Carolyn Rosenberg under the direction of Dr. T. W. Hastings of the Cornell Medical School.

The figures in Table 2 illustrate the manner in which the cells were divided and are taken from persons representing about the mean.

The work above reported shows an increase of 22 per cent. in mononuclear cells and of 22 per cent. in red blood-corpuscles at this altitude over the same components at sea-level.

The increase of 6 per cent. in the total white blood-cells at this altitude may be without significance, yet it may possibly prove to be a new observation. For many years it has been known that a temporary leukocytosis occurs in the blood of persons arriving at a high altitude.

The attempt was made to differentiate the smallest lymphocyte cells with very scant protoplasm surrounding the nucleus, from the larger ones with more abundant protoplasm.

These distinctions, however, seemed to vary according to the thinness with which the films were spread, and the larger lymphocytes could not easily be distinguished from the smaller, when the smears were not exceedingly thin. The figures indicate, however, that these distinctions were attempted, and they seem to prove that it is the larger lymphocytes of the mononuclear elements that are increased at this altitude.

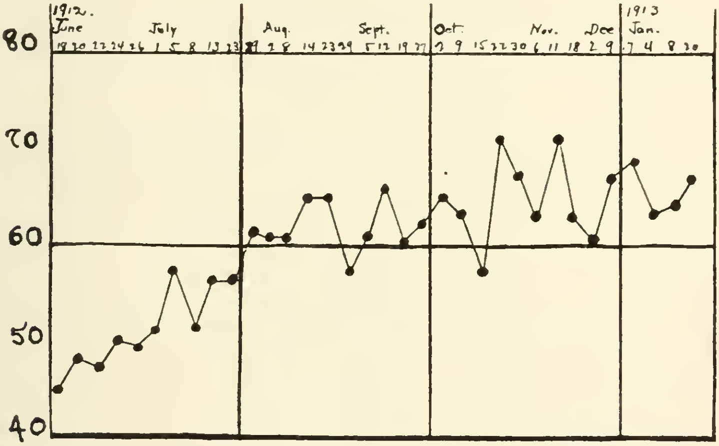

Chart 1.—Composite curve of the differential leukocyte count in twelve healthy *Macacus rhesus* monkeys brought from New York City to Colorado Springs, June 17, 1912. The counts of a monkey which broke its leg July 1 are omitted after that date; the counts of another in which tuberculosis was proved October 2 are not included after that date.

On June 17, 1912, the arrival of twelve healthy *Macacus rhesus* monkeys from New York City gave us further opportunity for an accurate observation of the possible influence of the change in altitude on the differential leukocyte picture. Films were taken from all monkeys every few days for a period of several months. The blood was obtained from the ear vein at noon when the animals were fasting. The total leukocyte count remained practically stationary during this period.

TABLE 2.—Distribution of Cells in Persons Representing About the Mean

	Poly-morpho-nuclears	Very Small Lympho-cytes	Larger Lympho-cytes	Large Mono-nuclears	Trans-itionals	Total Pct. Mononu-clear Element	Eosin-ophils	Mast	Total Leuko-cytes per c.mm.	Total Mononu-clears per c.mm.
New York	63.0	22	3	5.0	4.0	(34)	2⅔	⅓	7,000	2,380
Colorado Springs..	54.5	7	29	1.5	5.5	(43)	2.5	0	7,390	3,169

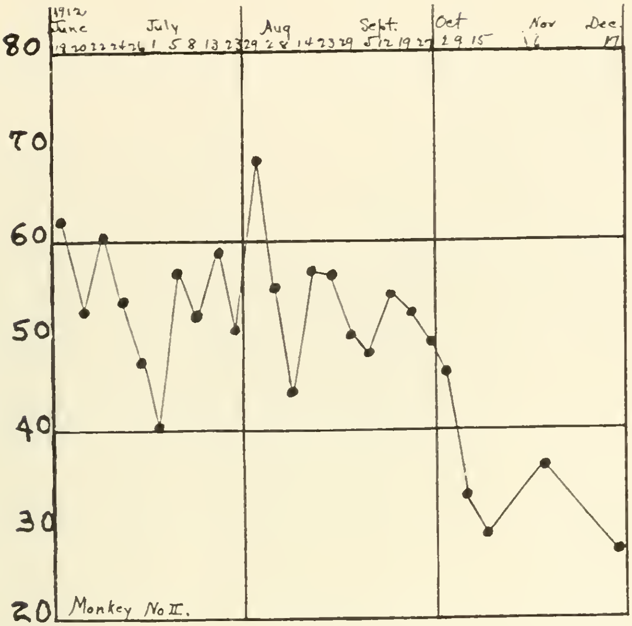

Chart 2.—Percentage of change in the mononuclear elements in a tuberculous monkey.

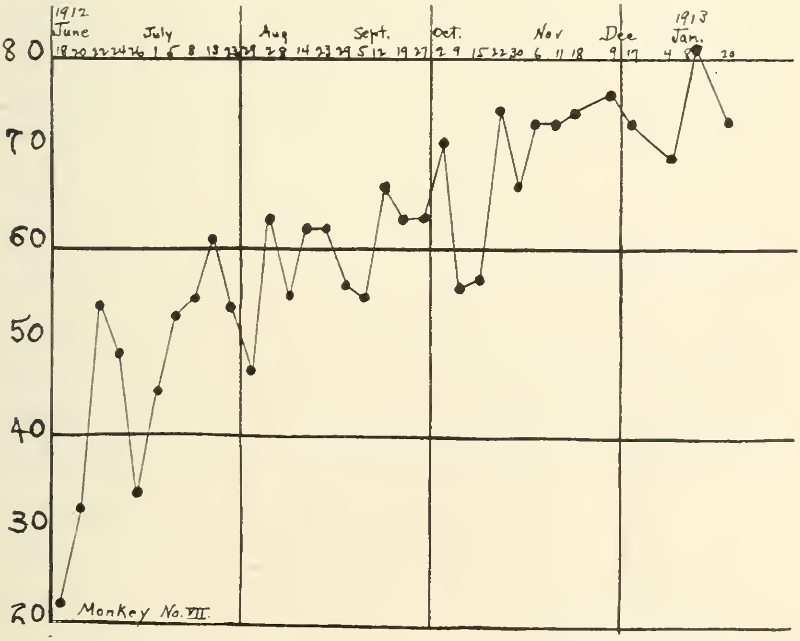

Chart 3.—Typical rise in the percentage of lymphocytes of a normal monkey.

Chart 1 is a composite showing the average percentage of mononuclear cells on the dates given. The increase from about 50 per cent. to 65 per cent. shows a percentage increase of thirty, and represents a very definite rise in the total numbers.

The counts on Monkey 4 are not included in this average after July 1, on account of its leg having been broken. The counts on Monkey 2 are also not included after October 2 when tuberculosis was suspected and confirmed.

Chart 2 illustrates the percentage of change in the mononuclear elements resulting from tuberculous infection in this monkey. The probability is that this monkey was infected on arrival, although the tuberculin test at that time was negative. Oct. 14, 1912, after the large decrease in the percentage of lymphocytes was noticed, a tuberculin test was found to be positive. The animal was killed Jan. 29, 1913 and showed extensive tuberculosis.

Chart 3 delineates the typical rise in the percentage of lymphocytes of a normal monkey.

CONCLUSIONS

1. It is certainly safe to assert that at an elevation of 6,000 feet the larger lymphocytes are absolutely increased in the circulating blood by at least 20 or 30 per cent. in both man and monkey.

2. The basophilic mononuclear elements in the blood of man at sea-level are approximately 34 per cent., and at 6,000 feet are about 42 per cent.

3. The total white blood-cells per cubic millimeter are approximately the same at sea-level and at an altitude of 6,000 feet, namely, about 7,500.

4. The total polymorphonuclear cells diminish in the exact proportion in which the mononuclear cells increase.

5. The red corpuscles increase by 22 per cent. at an altitude of 6,000 feet.

Our thanks are due to Dr. G. Burton Gilbert and Dr. T. W. Hastings for their kind supervision of this work.

This work has been aided by a grant from the American Medical Association to Dr. Gerald B. Webb. The authors are also under obligations to Mr. and Mrs. B. C. Allen and Mr. Richard F. Howe of Colorado Springs for their generous support.

THE GLYCYL-TRYPTOPHAN REACTION IN MENINGITIS *

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The presence of a proteolytic ferment in certain pathological exudates has long been known, and an examination of the literature discloses numerous investigations on the subject. Among these may be mentioned the work of E. Müller,[1] who, by the use of serum plates, showed the marked proteolytic action of pus cells. In the course of his investigations he also studied various exudates and transudates and found that normal cerebrospinal fluid contained no proteolytic ferment. In the cerebrospinal fluid of purulent meningitis he demonstrated the presence of ferment which digested the serum plates; in tuberculous meningitis no digestion occurred.

More recently Lenk and Pollak[2] have found the presence of a proteolytic ferment in certain exudates and transudates. They used glycyl-tryptophan as an indicator for determining the presence or absence of such a ferment. Glycyl-tryptophan, as is well known, first came into prominence through the publications of Neubauer and Fischer[3] who, following the work of Emerson,[4] which showed the presence of a proteolytic ferment in the gastric juice in carcinoma of the stomach, suggested it as a method of diagnosis. Subsequent investigations, however, especially those of Warfield[5] have shown the unreliability of this test. Warfield showed that sputum was able to split up the glycyl-tryptophan, and in addition to this peptid-splitting power of the sputum previous studies had already demonstrated the similar action of blood and bile.

Lenk and Pollak were especially impressed by the strong proteolytic or peptid-splitting powers of the cerebrospinal fluid in cases of tuberculous meningitis. In the normal cerebrospinal fluid they occasionally found such a ferment present in small amounts, whereas in tuberculous meningitis they found it present in large quantities and the fluid was usually able in dilutions of 1 to 100 or 1 to 200 to split the glycyl-tryptophan. In epidemic meningitis there was also a

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* Submitted for publication May 9, 1914.

* From the K. K. Kinderklinik, Vienna.

1. Müller: quoted by Lenk and Pollak (Note 2).
2. Lenk and Pollak: Deutsch. Arch. f. klin. Med., 1913, cix, 350.
3. Neubauer and Fischer: Deutsch. Arch. f. klin. Med., 1909, xcvii. 499.
4. Emerson: Deutsch. Arch. f. klin. Med., 1901, lxxii, 415.
5. Warfield: Johns Hopkins Hosp. Bull. 1911, xxii, 150.

marked increase in the amount of the ferment, but dilutions higher than 1 to 10 or 1 to 20 failed to show any peptid-splitting action. In meningismus, often simulating true meningitis, they found no increase. Their material includes 8 cases of tuberculous meningitis, 5 cases of epidemic meningitis and 7 cases of meningismus.

Koch,[6] in a more recent publication, did not find the high values described by Lenk and Pollak, but in 7 cases of tuberculous meningitis found the reaction positive in the undiluted cerebrospinal fluid of all, although in 1 case only, was a dilution as high as 1 to 10 positive.

Mandelbaum,[7] working with cerebrospinal fluid obtained from cadavers, has confirmed the peptid-splitting powers in cases of tuberculous meningitis and reports some high dilutions of the cerebrospinal fluid having this power.

The advantages of glycyl-tryptophan in studying the peptolytic action of various exudates and transudates are quite obvious. It is a fairly stable polypeptid, but under the action of peptolytic ferments, tryptophan is split off whose presence is easily recognized. By acidifying slightly the solution to be tested and then adding a few drops of chlorin water or bromin water, a red color-reaction is obtained with tryptophan.

We were able during the winter of 1912-13 at the time of Lenk and Pollak's preliminary communication, to test out the reaction on the cerebrospinal fluid in 17 cases of meningitis and in 12 cases of other diseases in which a complicating meningitis was suspected. The results of these tests are given in the accompanying tables.

We were not so much interested in the question as to whether the glycyl-tryptophan test allowed the conclusion that the fluid examined was a transudate or an exudate, nor were we attempting to solve the question of the suspected fluid being tuberculous or non-tuberculous. Our primary object was to determine whether this test, so simple of application, was of practical value in making a probable diagnosis possible in certain obscure cases with meningeal symptoms. Also to see if this test aided us in clinching the diagnosis of an already fairly certain meningitis.

The carrying out of the test is quite simple. One cubic centimeter of the cerebrospinal fluid to be examined is placed in a test-tube with an equal part of glycyl-tryptophan and then 1 c.c. of toluol added as a preservative. In order to test the peptolytic powers of the cerebrospinal fluid, various dilutions with sterile normal salt solution up to 1 to 200 are made. The mixture is placed in the thermostat for three hours. At the end of that time it is withdrawn, a few drops of dilute acetic acid added, and then very carefully a small amount of over-

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6. Koch: Ztschr. f. Kinderh. 1914, x, 1.
7. Mandelbaum: München. med. Wchnschr., 1914, lxi, 461.

saturated calcium chlorid which is briskly shaken just before using to give off free chlorin. It is safer to dilute the calcium chlorid 1 to 10 and it should be added drop by drop, as an excess of the solution masks the reaction. In the presence of free tryptophan a red color, as already stated, is found. Cerebrospinal fluid containing blood is of course to be rejected.

Here follow the tables of the cases in which these tests were applied. In these tables the highest dilution which was positive at any time is recorded, and the dilutions are indicated by the numbers set down, thus 10, 20, 50, refer to a dilution of 1:10, 1:20 and 1:50, respectively. The term "peptolytic index" which is subsequently used, refers to the dilution of cerebrospinal fluid which gave a positive reaction, thus "index 1" means undiluted fluid, "index 10" means a dilution of 1:10.

TABLE 1.—CASES OF MENINGITIS

No.	Age, Years	Clinical Diagnosis	Autopsy Findings	Positive Reaction	Negative Reaction			
1	2	Tub. men.	Tub. men.	20			50	100
2	2	Tub. men.	Tub. men.	200				
3	2	Tub. men.	Tub. men.	100				
4	8/12	Tub. men.	Tub. men.	100				
5	2	Measles, tuberculous meningitis	Tub. men.	5	10	20	50	100
6	1 8/12	Tub. men.	Tub. men.	50				
7	10	Tub. men.	Tub. men.	20	50	100	200	
8	2	Tub. men.	Tub. men.	50				
9	6	Tub. men.	Tub. men.	20	50	100		
10	1 1/12	Tub. men.						
11	2 1/2	Tub. men.	Tub. men.	1	10	20	50	100
12	7	Tub. men.	No autopsy	20				
13	4	Tub. men.	No autopsy	100				
14	4	Tub. men.	No autopsy	100				
15	1	Tub. men.	No autopsy	10	..	20		
16	5 1/2	Tub. men.	No autopsy	10	20	50		
17	1 1/2	Purulent meningitis (pneumococcus)	Purulent meningitis	1	10	20	50	100

TABLE 2.—NON-MENINGEAL CONDITION

No.	Age, Years	Clinical Diagnosis	Autopsy Findings	Positive Reactions	Negative Reactions				
1	9	Coma, meningitis?	Recovered	0	1	10	20	50	100
2	3½	Delirium meningitis?	Enteritis	0	1	10	20	50	100
3	7	Scrofula, coma, meningitis?	Brain abscess	0	1	10	20	100	
4	1½	Ant. poliomyelitis	Recovered	0	1	10	20	100	
5	2	Measles, miliary tuberculosis?	Recovered	0	1	10	20	50	100
6	12	Typhoid meningitis?	Recovered	0	1	5	10		
7	7*	Miliary tuberculosis	No autopsy	0	1	10	20	50	100
8	4	Measles, miliary tuberculosis?	Recovered	0	1	5			
9	4	Measles, miliary tuberculosis?	Recovered	0	1	5			
10	4	Scarlet fever	Recovered	0	1	10			
11	7	Scarlet fever	Recovered	0	1	5	10		
12	11/12	Hydrocephalus luetica	....	0	1	10	20	50	100

* Weeks.

It will be seen on glancing over Table 1 that while tuberculous meningitis usually gave a positive reaction in high dilutions, yet this was by no means always the case. In Case 11 dilutions of the cerebrospinal fluid contained no demonstrable peptolytic ferment; in Case 5, 1:5 was the highest dilution positive and in Cases 10, 15 and 16 dilutions higher than 1:10 were negative. There is, however, evidence in our series of cases that this proteolytic index may vary from time to time. Cases 3, 6, 9, 11, 14 and 16 showed these variations. Case 6 is a good example.

Case 6.—A. W. L., aged 20 months, male, admitted to hospital Feb. 21, 1913, with fever, rigidity of neck, stupor and unconsciousness. Clinical Diagnosis: Tuberculous meningitis.

The cerebrospinal fluid when tested for peptolytic ferment gave the following results in the various dilutions:

February 21—1+ 10 neg. 20 neg.
February 25—1+ 5+ 10+ 20 neg.
February 26—10 neg. 20 neg. 50 neg.
March 3—1+ 10 neg. 20 neg.
March 9—1+ 10+ 20+ 50+
Child died March 9. Anatomical Diagnosis: Meningitis tuberculosa.

Several cases have shown a gradually increasing peptid-splitting power of the cerebrospinal fluid as the disease progressed. Cases 1, 2, 4 and 8 exhibited this. Case 2 is a good example.

Case 2.—O. F., aged 2 years, male, was admitted to hospital Oct. 26, 1912, with fever, unconsciousness, strabismus and rigidity of neck. Clinical Diagnosis: Tuberculous meningitis. Tests with the cerebrospinal fluid gave the following results:

October 26—1+ 20+ 40 neg. 100 neg.
October 29—1+ 20+ 50+ 100 neg.
November 2—1+ 10+ 50+ 100+
November 3—50+ 100+ 200+
Death on November 3. Anatomical Diagnosis: Meningitis tuberculosa.

In several cases the reaction was of distinct value in clearing up an obscure diagnosis. Case 4 is an example.

Case 4.—L. K., aged 7, male, was admitted to hospital Oct. 30, 1912. Scrofulous child, Pirquet +. Coma, slow respirations, rigidity of neck. Cerebrospinal fluid showed no splitting of glycyl-tryptophan. Child died on day of admission. Anatomical Diagnosis: Brain abscess.

In none of the cases without meningitis was any splitting of the glycyl-tryptophan observed. Koch[6] reports one case of pneumonia with meningeal symptoms, and one case of sarcoma of the skull which gave positive reactions with undiluted cerebrospinal fluid. As the autopsy findings are not reported one is left in doubt as to whether there was an accompanying meningitis. Lenk and Pollak report several cases of meningismus in which positive reactions were found. In these cases autopsy showed the meninges to be free. From their series of cases it seems probable that the undiluted cerebrospinal fluid in non-meningeal conditions may sometimes split glycyl-tryptophan. The study of a large series of normal cerebrospinal fluids would be desirable in order to settle this point definitely.

Far-reaching conclusions we do not wish to draw from the results in our cases. But we do feel that in glycyl-tryptophan we have another valuable addition to our diagnostic measures in meningitis. In cases having a peptolytic index higher than 1 we have consistently found meningitis present. Only one case of tuberculous meningitis showed an index as low as 1. The striking variations in peptolytic powers which were observed from time to time makes it probable that cases showing a low index might at some time have shown a much higher one. Also there was noted an interesting tendency for the peptolytic powers of the cerebrospinal fluid to increase as the disease progressed.

ELLIPTICAL HUMAN ERYTHROCYTES *

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The following observations are published in view of the rarity of the condition, and because of the interesting discussion which followed the report of the only other case on record.

April 14, 1913, J. F., male, aged 41, was admitted to the surgical service of Dr. Henry H. M. Lyle at St. Luke's Hospital, New York City, with the diagnosis of acute suppurative appendicitis. According to the routine procedure of the hospital a leukocyte count was made. While examining a stained smear to determine the differential count I noticed that many of the red blood-cells were not circular but elliptical. So striking was this poikilocytosis that in spite of the absence of erythroblasts, a diagnosis of pernicious anemia seemed to be warranted. An estimation of the hemoglobin, however, showed 110 per cent. (Fleischl-Miescher), and of the red blood-cells, 5,400,000.

The patient gave a typical history of acute appendicitis. No lesions of heart, kidneys or lungs were discoverable. Examination of his abdomen revealed tenderness and rigidity of the right lower quadrant. His temperature was 102 F., his leukocytes were 16,500, polynuclears, 82, lymphocytes, 18. Urine, acid, turbid, 1.015, albumin very faint trace, sugar, 0, few leukocytes. Operation was performed two hours after the patient entered the hospital and a gangrenous appendix with a small amount of seropurulent fluid free in the abdomen, was found. Recovery was uneventful, the temperature being normal on the seventh day after operation and the patient being discharged cured on the tenth day.

During his stay in the hospital a careful study of his blood was conducted. About 75 to 80 per cent. of the erythrocytes were observed to be elliptical in outline. The proportion between the long and short diameters of these cells varied considerably, some of them being only slightly elliptical, while others were very distinctly elongated. The following measurements may be interesting:

Circular cells measured 8.5 microns in diameter.
Greatest length observed, 13.7 microns.
Shortest length observed, 8.2 microns.
Shortest width observed, 5.0 microns.
Greatest width observed, 8.0 microns.
Most of the elliptical cells measured about 13.0 by 5.0 microns.

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* Submitted for publication, April 27, 1914.

* From the Pathological Laboratory of St. Luke's Hospital, New York, Prof. Francis Wood, director.

Every precaution was taken to exclude the possibility of mechanical distortion of the cells. Smears were made by several persons, and by several methods, and the slides were dried both slowly and rapidly, but the deformity always appeared the same. Blood diluted with Hayem's solution also showed the anomaly; and finally specimens of the fresh blood were examined and the cells were still found to be elliptical.

The fragility of the erythrocytes was determined and found normal. Vital staining showed only 3 per cent. reticulated cells—well within normal limits. The Wassermann reaction was negative.

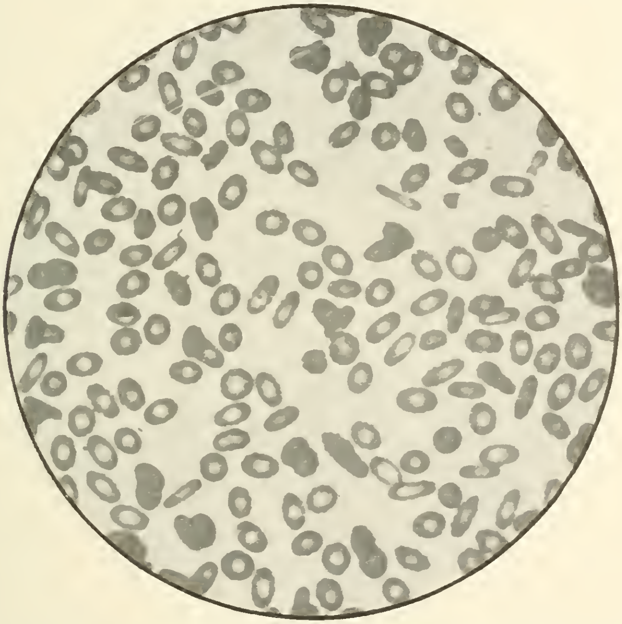

Fig. 1.—Blood from J. F., April, 1913, magnified 500 diameters, showing elliptical corpuscles. This and the Figures 2, 3 and 4 were all taken without changing lenses or apparatus, so that they are exactly the same magnification. The smears were made in the same manner.

The possibility of this condition being a congenital characteristic of the man's blood suggested itself. Working on this theory the blood of his sister was obtained and was found to show an exactly similar condition. Her red blood-cells were 5,000,000, her hemoglobin 100 per cent., and about 70 to 80 per cent. of her cells showed a more or less well-marked elliptical outline. A few of them showed a more extreme deformity than those of her brother. One cell was found to measure 17.2 by 3.2, and several were 15 microns long. As far as she could remember she had never had a sick day in her life.

The other members of the family—a second sister, her father and her two children (both boys)—were found to have normal erythrocytes; unfortunately the mother of my patient is not living. To exclude the possibility of some chronic poisoning by lead, coal-gas, or other chemical, the blood of all the persons living in the same house with this family was examined and found to be normal.

Mr. F. has been under observation for eight months and is apparently in perfect health, never having had any illness before or since his appendicitis. His blood has been examined six times during this period and has always shown the same unvarying deformity of the red cells. His hemoglobin and the number of his red cells have always been normal. The same facts are true of the sister's blood.

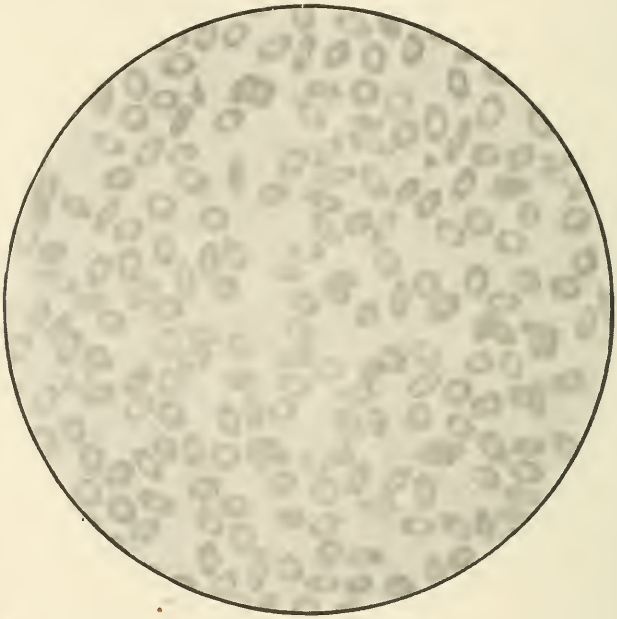

Fig. 2.—Blood from J. F., December, 1913, magnified 500 diameters.

The only record of a similar case which I have been able to find was that observed by Dr. Melvin Dresbach.[1] Dr. Dresbach reports that in 1902 a student working in the Ohio State University laboratory of histology discovered that his erythrocytes were elliptical in outline, 90 per cent. of the cells being deformed. Variously prepared and stained specimens and fresh blood were examined. His hemoglobin and the number of his red cells were normal. The case was studied over a period of four months and always showed the same characteristic. The following are the measurements given for this man's blood:

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[1] Dresbach, Melvin: Science, 1904, xix, and 1905, xxi.

Shortest width observed	3.9 microns
Greatest width	4.8 microns
Shortest length	8.5 microns
Greatest length	10.7 microns
Average length	10.3 microns
Average width	4.1 microns

I have examined a photomicrograph of this blood and the picture is much the same as in J. F., but the amount of deformity is slightly greater.

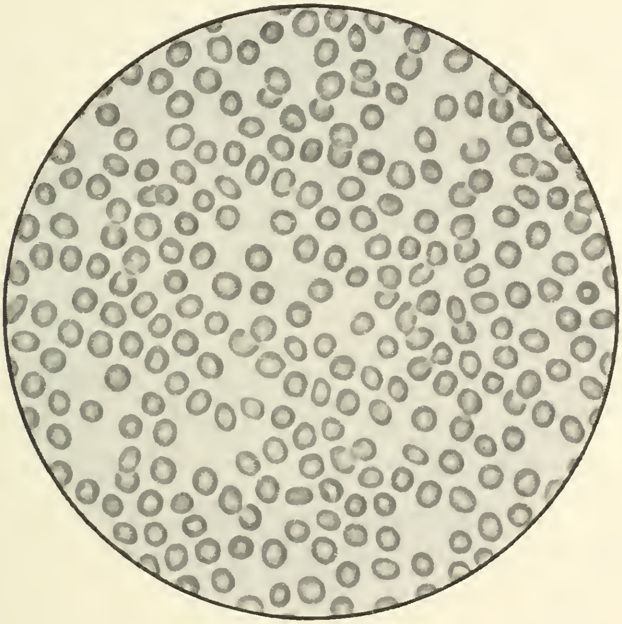

Fig. 3.—Normal blood magnified 500 diameters.

The student was a healthy mulatto, aged 22 years. Dr. Dresbach says:

> The observation attracted considerable attention, Prof. Austin Flint being one among several who wrote to me for a specimen of the blood. There resulted some correspondence between Professor Flint and myself, and in a letter to him I remarked that some time after my observations had been made the young man having these elliptical corpuscles died. Since the claim had been made that the young man was healthy when these corpuscles were discovered, the statement about his death naturally aroused some suspicion. Professor Flint wrote a note which was printed in *Science,* May 20, 1904, discussing the matter of form changes in erythrocytes and pointing out that in progressive pernicious anemia and other blood diseases, variations in form and size are well known facts. He adds that in view of these facts it seems impossible to accept the proposition that the subject of the observation was a healthy mulatto. The cause of death was cardiac failure subsequent to an attack of

acute inflammatory rheumatism. The attack was preceded by tonsillitis, which began about three months after the time observations were made.

Prof. James Ewing of the Cornell Medical School, saw the blood specimen and was of the opinion that the anomaly was one of congenital or developmental origin—because the hemoglobin and number of red cells was normal and the patient's general condition good.

The slides were also shown to Prof. Paul Ehrlich, who thought that these cells showed a congenital anomaly. Dr. Dresbach gives the following reason for agreeing with these opinions, and I think they apply equally to my cases.

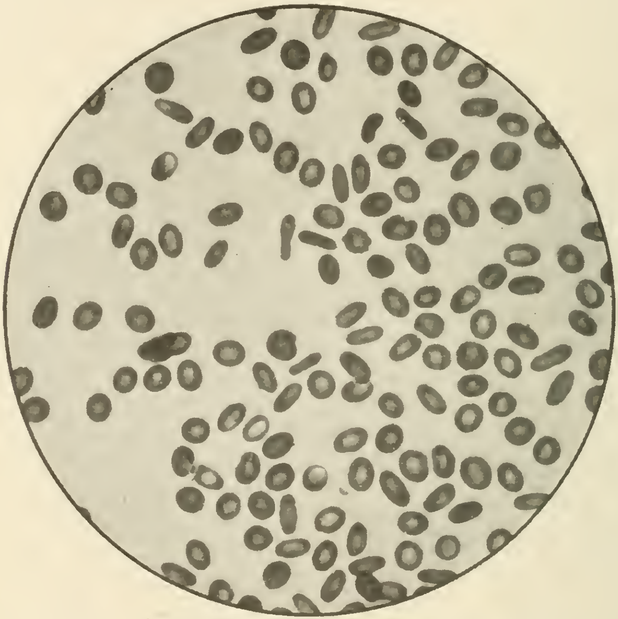

Fig. 4.—Blood from a sister of J. F., December, 1913, magnified 500 diameters.

The variation occurred in a healthy individual. Good general physical condition, normal number of red and white cells, normal hemoglobin and absence of normoblasts or megaloblasts preclude the idea of any known blood disease being connected with the phenomenon. In Dr. Dresbach's case no family history could be obtained except that a brother of the patient had normal corpuscles. The fact that in my own case two members of the same family have been observed to show this remarkable cellular deformity, I think is very strong evidence in favor of the theory that the condition is a congenital anatomical defect.

St. Luke's Hospital.

THE PITUITARY GLAND IN EPILEPTICS *

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SONYEA, N. Y.

It seems probable that the time is past when we can group as due to a single cause or group of causes the numerous nosological types which in the past we called epilepsy, but which to-day we call the epilepsies, or epileptic syndromes. Our efforts, if they are to be ultimately profitable, must now be directed to the clinical study of the disease and to the construction of an adequate classification. Even in the smaller groups into which we shall divide the epilepsies, we shall find, in each, the result of the interaction of many factors, differing more or less in the individual case; in fact, the epilepsies are to be regarded as due to a summation of causes, rather than as a manifestation of some one single etiological factor.

At present, epilepsies are generally classed as symptomatic and idiopathic (also genuine or essential). In the latter group are placed all cases in which there is no obvious anatomical lesion or extrinsic condition at the bottom of the disease. Considerable time and space have been devoted to theorizing on the etiology of this residual group of epilepsies, and among the most attractive of the suggestions made is that of disturbed metabolism, especially disturbances of the glands of internal secretion.

The necropsy material available at the Craig Colony represents a considerable opportunity for the study of anatomical conditions associated with the disease, and we have for some time been collecting material which might cast some light from an anatomical point of view on the conditions of the ductless glands in epilepsy. The thyroid and pituitary glands were the only ones showing any great change in appearance; both of these showed more or less variation from what might be considered an average or normal type. In this paper we shall deal with the anatomical conditions found in the pituitary gland of epileptics and with certain clinical findings which may be of interest in connection with the rôle played by this gland.

ANATOMICAL FINDINGS

The sella turcica has not as a rule been enlarged; ordinarily, the gland has completely filled it. There has, however, been a distinct group of cases in which there has been a marked difference between the size of the sella and the size of the gland. In these cases the dura

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* Submitted for publication, Jan. 20, 1914.

is seen pouched down into the sella, instead of the more usual flat membrane between the clinoid processes. When this pouching is found, the gland may be flattened (scaphoid) in shape, but small glands or large fossae may also account for the condition in different cases. In only one such case have we taken measurements of the sella; they were as follows: between anterior clinoid processes, 28.3 mm.; between posterior clinoid processes, 17.5 mm.; between optic foramina, 19.8 mm.; from optic foramen to posterior clinoid process, 15.5 mm.; width in sagittal plane, 15.5 mm.; depth, 16.6 mm. The gland itself in this case (A 416) measured 17.5 by 15.5 by 4.7 mm. The woman

TABLE 1.—Dimensions and Weights of Pituitary Glands

Necropsy No.	Width (Lateral) cm.	Ant.-Post. cm.	Thickness cm.	Weight gm.
390	1.60	1.05	0.55	0.5938
392	1.42	0.966	0.645	0.560
393	1.04	0.775	0.888	.....
395*	1.20	0.7	0.5	0.281
396	1.10	0.9	0.5	0.345
398	1.50	1.4	0.3	0.603
400	1.40	1.0	0.6	0.504
401	1.5	0.9	0.6	0.586
403	1.20	0.8	0.5	0.504
404	1.50	1.1	0.4	0.454
405†	1.65	1.2	0.7	1.032
406	1.40	1.1	0.7	0.61
407	1.3	1.0	0.5	0.428
Average...	1.37	0.98	0.56	0.541
High.......	1.65	1.40	0.88	1.032
Low.......	1.04	0.7	0.3	0.281

* Smallest. † Largest.

TABLE 2.—Additional Gland Measurements

Necropsy No.	Width cm.	Ant.-Post. cm.	Thickness cm.	Weight gm.
416	1.75	1.55	0.47	....
421	1.50	0.85	0.55	0.51
422	1.40	0.80	0.60	0.410
423	1.60	1.05	0.55	0.612
424	1.30	0.90	0.55	.....
425	1.20	1.00	0.60	0.489
426	1.50	1.30	0.50	0.620
427	1.60	1.10	0.65	0.735
428	1.30	1.10	0.60	0.480
Average.....	1.46	1.07	0.56	0.551

in whom this was found was decidedly fat; ovaries small and fibrous, uterus unicornus; age at death 44 years. Also, in a case of internal hydrocephalus, a much-pouched dura was found. It is not cases, however, in which increased intracranial tension is apparently present that are interesting, but those in which the pouching is without apparent cause other than a disparity between the bony fossa and the gland.

We regret that we have not a larger series of weights and measurements of pituitaries to report. A small series were measured at the colony by Mr. Worthing of the 1913 medical class of Syracuse University, and a few additional have been recorded (Tables 1 and 2).

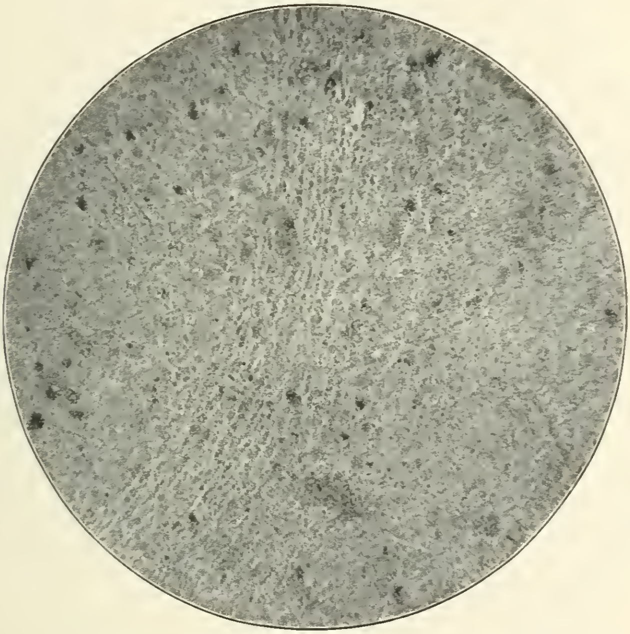

Fig. 1.—Case A-356. These figures show large adenomatous area in the pars glandularis, no symptoms noted.

Dock[1] quotes Zander as giving the size of the pituitary gland as variable from 6 to 10.5 mm. in sagittal, from 10 to 14.5 mm. in vertical and from 5 to 9.75 mm. in transverse diameter, and weighing 0.6 gm. Our figures are within this range, though the weight tends to be lower in our series.

Our figures compare somewhat unfavorably with the dimensions and sizes given by Biedl:[2] anteroposterior, 14.4 mm.; lateral, 21.5 mm.; thickness, 5.5 mm.; weight, from 0.59 gm. to 0.731 gm. (as great as

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1. Dock: Modern Medicine (Osler) Lea and Febiger, 1909, vi, 463.
2. Biedl: Innere Sekretion, Urban and Schwarzenberg, Berlin, 1910, p. 281.

1.65 gm. in multiparas). The twenty-two glands recorded in this paper appear to be somewhat below the average of these figures.

No large *tumors* of the pituitary have been found at the colony, but in three cases large tumor-like structures have been found. There were no clinical symptoms in any of these cases, and the detailed case notes will therefore not be given; it should be stated that the condition was not suspected, and thus significant clinical conditions may have escaped notice. Figures 1, 2, 3 and 7 show the nature of the process in each case.

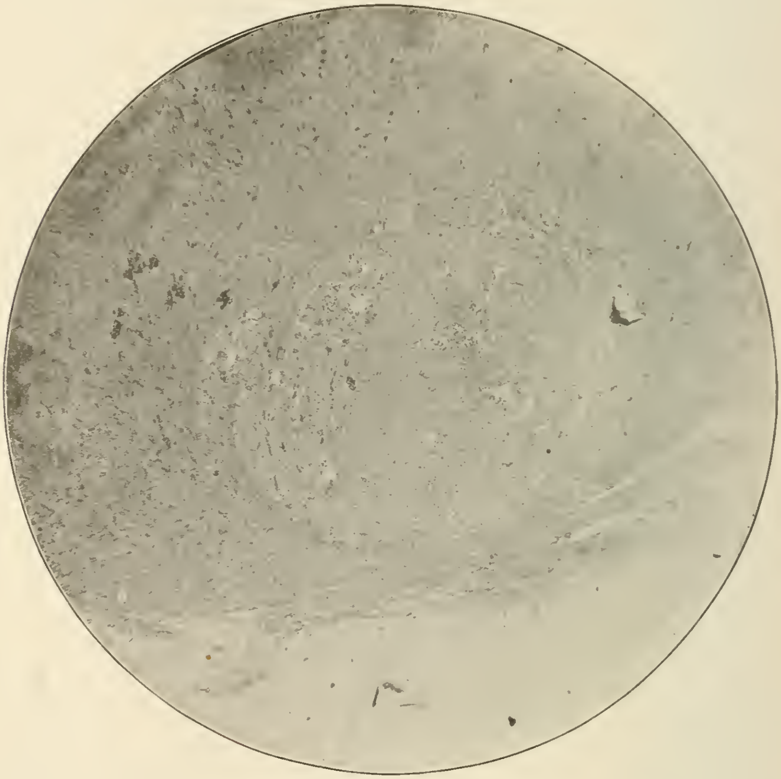

Fig. 2.—A-295. Adenomatous area in pars glandularis. No clinical data.

One of the most striking variations in the appearance of sections of the pituitary is variation in the blood-content of the gland. Certainly, in many cases it considerably exceeds the normal. All degrees are present and an uneven distribution of the congestion is not uncommon (Fig. 4).

No particular cell type can be said to predominate in the glands examined in our series. There are occasionally glands in which the cyanophil cells are considerably more common than the average.

The ordinary illustrations and descriptions of the pituitary do not show much connective tissue[3] in the pars anterior. The acini seem normally to be supported by a very slender framework, but in the cases examined at the colony, increases of connective tissue seem to be decidedly common. These are of two types, diffused and localized.

As shown in the photomicrographs (Figs. 5 and 6), there are found extremely irregular spider-like masses of connective tissue in the middle of the pars anterior. These are made up of dense hyaline

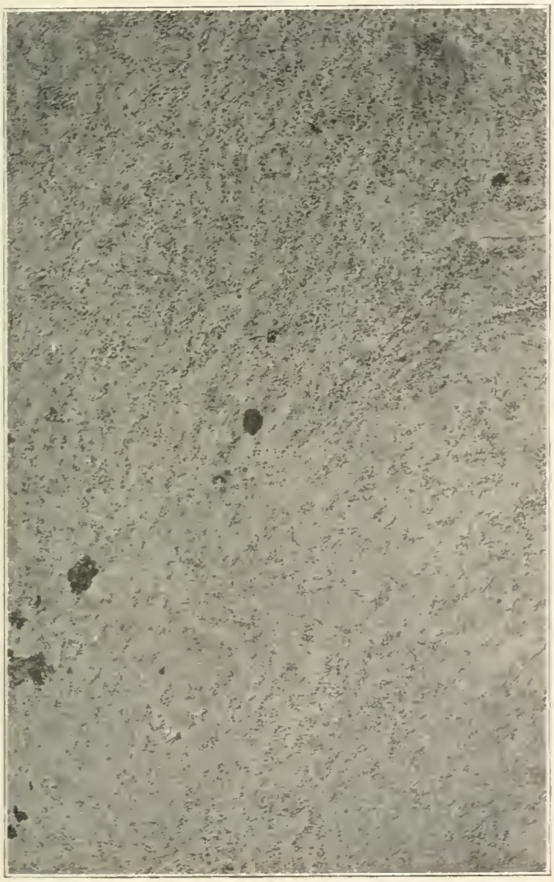

Fig. 3.—Case A-405. Section of "tumor" showing that it is composed of bands of dense connective tissue, markedly fibrous, between which are acini, narrowed and compressed. This patient also had a glioma of the temporosphenoidal region.

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[3] Claude, H., and Schmiergeld, A.: (L'encéphale, Jan. 10, 1909, abstracted in Epilepsia, ii, 207), say that the hypophysis often presents sclerosis, etc. Cushing, in a personal communication, also speaks of the great variety of changes which may be found in the gland.

tissue, with no great amount of fibrillar structure. Within them, in the central portion, are blood-spaces, and from the borders, irregular trabeculae are sent out which gradually break up and disappear between the acini. Near the central mass, these trabeculae often enclose acini, and the latter are apt to show an excess of cyanophil cells. These "spiders" or "islands" tend to be located in the center of each lateral half of the pars anterior; sometimes only one is present. As a rule, these structures seem to have no connection with the capsule of the gland, but in a few instances there has appeared to be a connection extending toward the hilum of the gland, if we may so designate the hollow in which the pars posterior is placed; in one case, this connection was quite definite and showed indications of canalization by blood-spaces.

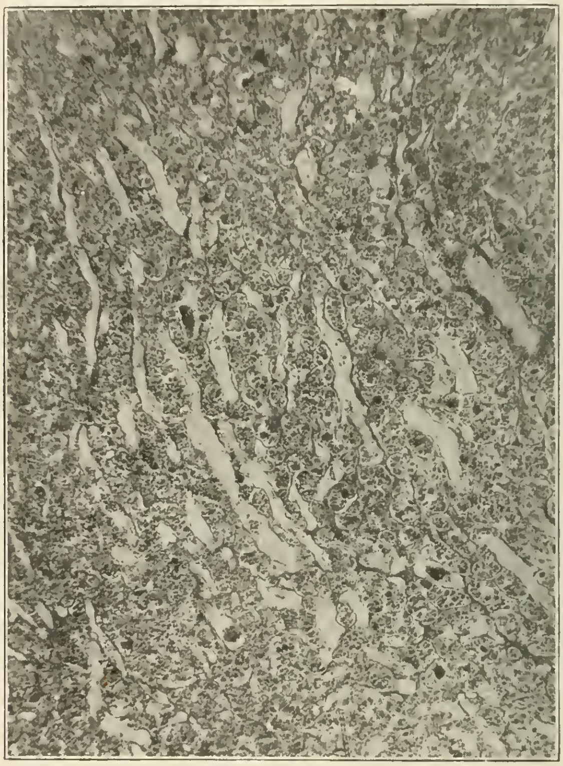

Fig. 4.—Case A-322. Enlarged blood-spaces.

The significance of these islands is not clear. They are not invariably found, though this may be due to failure to examine sufficient sections from a particular gland. It seems probable that they are associated with the blood-supply of the pars anterior, and that they may be residues from the ingrowth of connective tissue during fetal life, described by Frazer.[4]

The diffuse type (Fig. 7) of connective-tissue change is distinctly different and consists of a more or less extended thickening of the interacinar connective tissue. Ordinarily, composed of a few almost invisible fibers, the fibrous tissue between the acini may become almost as thick as the acinus is broad. Not all these appearances, however, are genuine diffuse change. If the reader will examine Figure 5, and imagine a section made close to the island, he will see that the section would have the appearance of connective-tissue increase between the acini, on account of the trabeculae coming from the island which has been cut across. The island, of course, would not show. Serial sec-

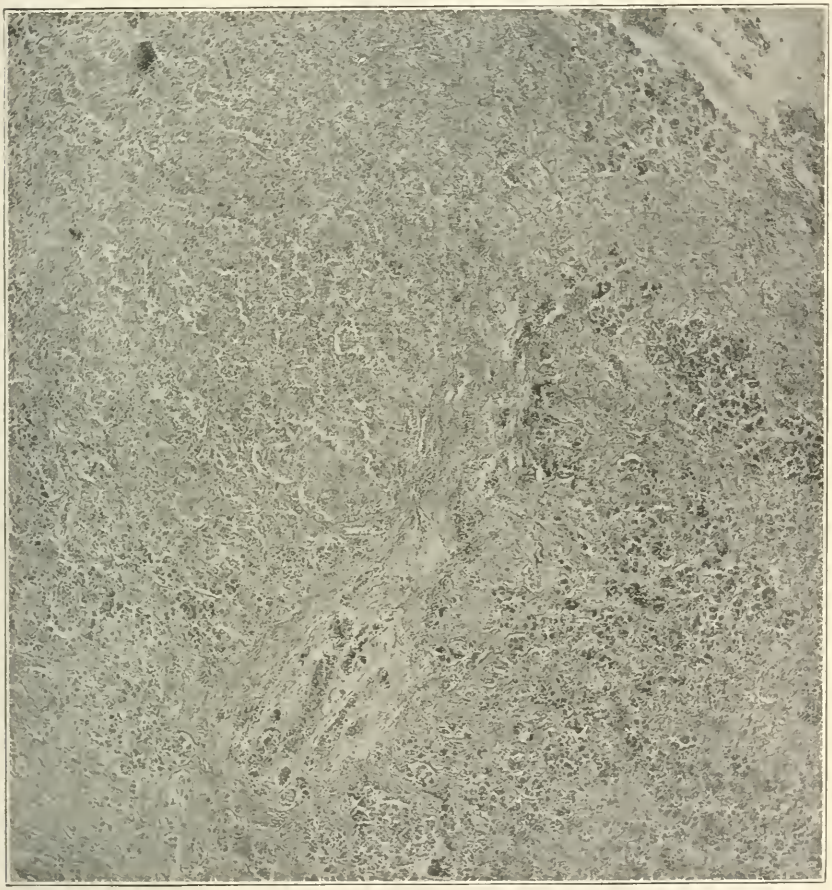

Fig. 5.—Case A-373. "Islands" of connective tissue.

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[4] Frazer: Lancet, London, Sept. 28, 1912.

tions, or at least a goodly number of sections from each gland, are necessary to the proper examination of this gland.

There is also a distinct tendency toward an increase of connective tissue in the region between the neurogliar and glandular portions. This is shown in Figure 8.

It is of interest that the brothers Wenzel (quoted both by Biedl and by Cushing[5]) looked on colloid collections in the pituitary as the cause of epilepsy. Colloid is almost always present in our glands, but only occasionally to any extent. These accumulations are usually in the region between the parts of the gland, though occasionally colloid acini are seen in the pars anterior (Figs. 8 and 9).

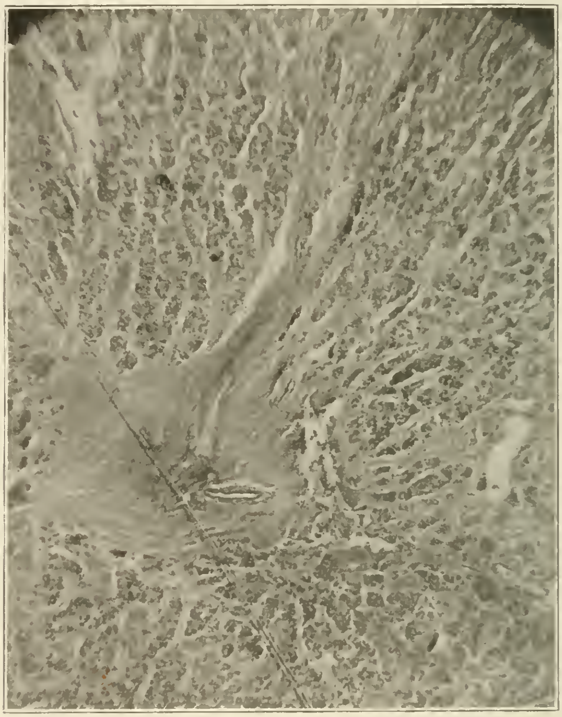

Fig. 6.—Case A.-423. "Islands" of connective tissue.

It will be seen that there are no constant changes in the pituitary gland from an anatomical point of view which have not been elsewhere reported; the connective-tissue "islands" are apparently an exception to this, but their significance is not clear — they are probably connected with the blood-supply. The possibly diminished size of the gland and an occasional large sella turcica appear the most striking signs of pituitary change; the tumor-like structures are of unknown significance in the absence of clinical symptoms.

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[5] Cushing, Harvey: The Pituitary Body and Its Disorders, Philadelphia, J. B. Lippincott Co., 1912.

CLINICAL DATA: BLOOD-PRESSURE AND SUGAR TOLERANCE

Cushing[5] advances the hypothesis that certain cases of epilepsy are associated with insufficiency of the pituitary gland. While it is not new to have the glands of internal secretion indicated as of importance in the production of epilepsy, the designation of a particular one and the definite characterization of its rôle as one of insufficient activity, rather than of perverted activity, and the presentation of a quite definite clinical picture of the condition, make Cushing's hypothesis worthy of careful consideration.

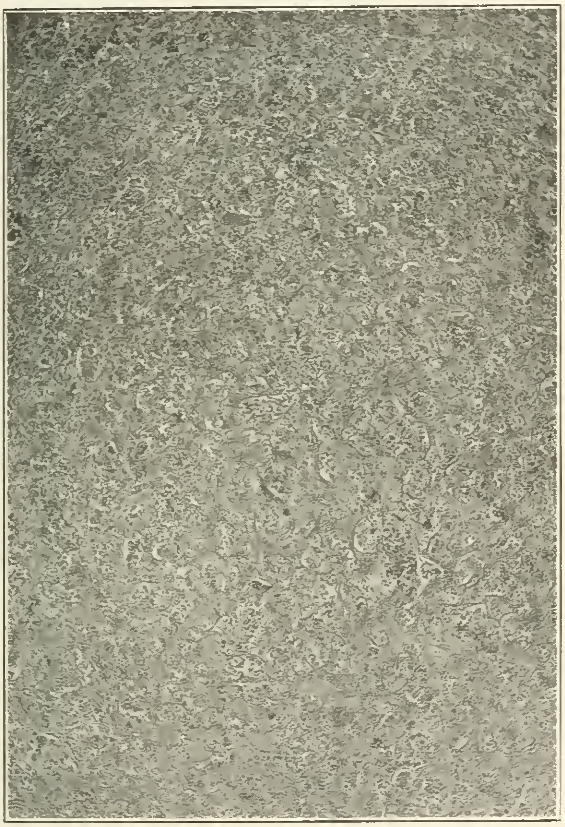

Fig. 7.—Case A-373. Diffuse interacinar connective tissue.

He sums up the basis for his hypothesis as follows:

1. Horsley found that the cortex of hypophysectomized dogs was more sensitive to stimulation than that of normal animals.

2. In a number of Cushing's hypophysectomized animals, a tendency to convulsions was observed.

3. Epilepsy is a frequent sequel of cranial injuries, and in certain of these, the pituitary is damaged.

4. Epilepsy is a frequent accompaniment of hypopituitarism.

5. The secretion of the posterior lobe apparently enters the cerebrospinal fluid and thus reaches the brain. Thus a scar or tumor, etc., may prevent the contact of the secretion of the posterior lobe with the nerve-cells, with resulting alteration in their stability.

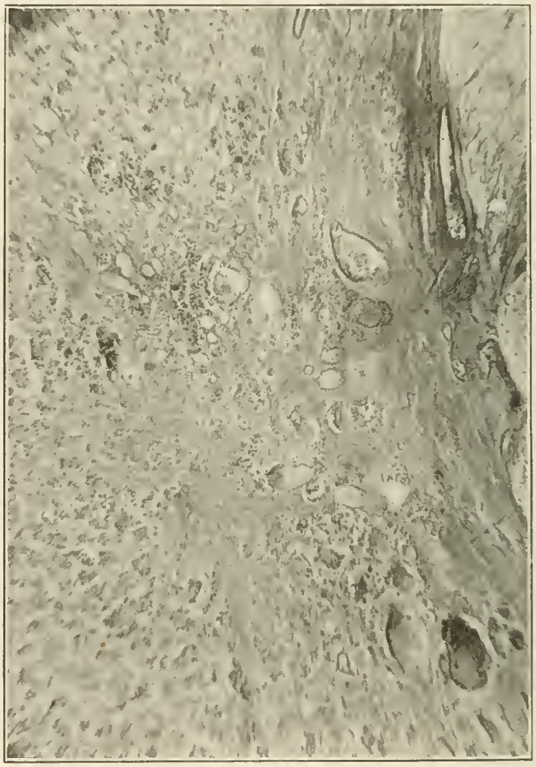

Fig. 8.—Case A-423. Amount of connective tissue sometimes seen between the two portions of the gland. Some of the rounded spaces contain colloid.

6. Many epileptics present symptoms of hypopituitarism, and administration of the gland substance modifies the seizures.

Cushing apparently offers four cardinal symptoms of states of pituitary hypoactivity: (1) high sugar tolerance; (2) subnormal temperature, slowed pulse and low blood-pressure; (3) drowsiness, and (4) asthenia.

Of the characters indicated by Cushing, subnormal temperature has been very frequently observed in epileptics, especially when combined with low mental grade and the accompanying asthenia. Reuben quotes from the literature that hypophysectomized dogs may have a temperature of 20 C. (68 F.) just before death. We believe that it may be accepted that subnormal temperatures are not uncommon among epileptics.

The literature contains a few references to blood-pressure in epileptics. Spratling[6] reports for thirty-three cases between 10 and 43 years of age, readings of from 115 to 135 mm. of mercury. Turner,[7] taking from 110 to 130 mm. as the normal average, reports that he found one epileptic with high and four each with normal or low blood-pressures in nine cases. Besta[8] reported 63 per cent. of his cases as having higher than normal blood-pressure. No data are given as to

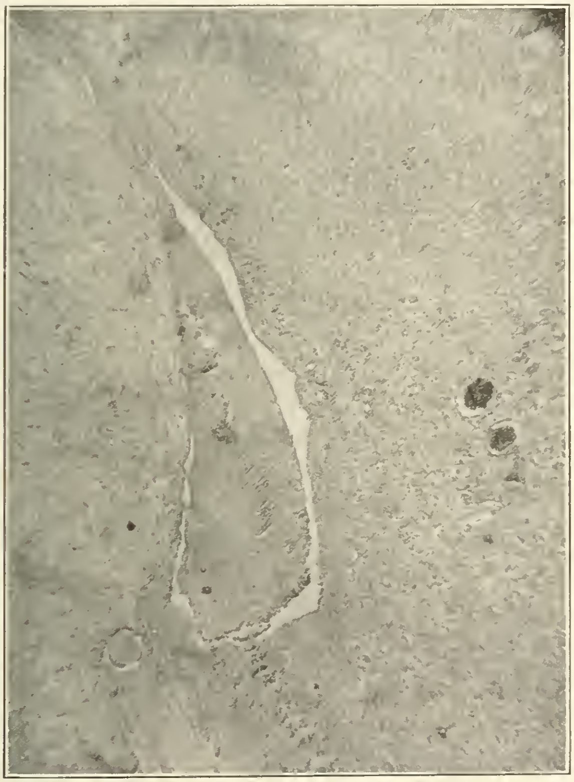

Fig. 9.—Case A-336. Colloid accumulation in a cleft between the two parts of the gland.

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[6] Spratling, W. P.: Epilepsy and Its Treatment, Philadelphia, W. B. Saunders & Co., 1904, p. 172.

[7] Turner: Jour. Ment. Sc., 1907, liii, 793.

[8] Besta: Jour. Ment. Sc., 1907, liii, 404.

the number of cases examined or his normal standard. Pollock and Treadway[9] report forty-one cases, of which eighteen had a systolic pressure of 120 or below and twenty-three ranged from 125 up to 200. From these rather meager reports it would seem that there was no striking change in the blood-pressure of epileptics as a class.

Our own series bears this out. Our results, using a group of sixty-six epileptic imbeciles, are given in Table 3. Two cases in the 12-to-20 year group, and one in the 31-to-40 group, were excluded from the averages on account of the excessively high figures which they showed.

TABLE 3.—Pressure by Ages

Year Group	Systolic	Diastolic	Pulse-Pressure	Cases
To 20 inc.	115	76	37	15
30	126	82	41	27
40	127	78	43	13
50	132	92	40	7
59	197	107	87	1

Grouped by the systolic pressure, the cases arrange themselves as follows:

Below	100 mm., 1		To	140 mm., 13	(19.60 per cent.)
To	110 mm., 4		To	150 mm., 6	
To	120 mm., 17	(25.75 per cent.)	To	187 mm., 1	
To	130 mm., 23	(34.85 per cent.)	To	197 mm., 1	

It is thus seen that low blood-pressures are rather uncommon and that in general the pressures observed agree with the physiological normals.

The diastolic pressures ranged as follows:

From 21 to 30 mm., 7	51 to 60, 9
From 31 to 40 mm., 24	61 and up, 2
From 41 to 50 mm., 23	

In a few cases we have observed the effect on blood-pressure of doses of pituitary whole gland extract by mouth. The changes are not at all characteristic and are influenced by the difference in the time of year — the control observations having been made during cold weather and these while under treatment in warm weather. Reversing the expectation based on the known pressor influence of pituitary extracts, the more striking change is a slight lowering of the blood-pressure or no change of blood-pressure. This portion of the work we do not regard as at all accurate, on account of the seasonal changes and the short periods of administration of the gland.

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[9] Pollock, L. J., and Treadway, W. L., A Study of Respiration and Circulation in Epilepsy, The Archives Int. Med., 1913, xi, 445.

The question of sugar tolerance in epileptics is one of more difficulty. The literature contains comparatively little of assistance as to either the normal or the pathological side.

The normal assimilation limit is variously stated, but appears for dextrose to be from about 100 to 300 gm., or a possible average of 2.5 gm. dextrose for each kilogram of body-weight. There are, however, factors which influence the assimilation limit and which are hard to control and estimate:

THE RATE OF INTRODUCTION AND ABSORPTION OF THE SUGAR

The kind of sugar influences this as well as the physical characters of the solution in which the sugar is administered. For absolutely accurate results it would be desirable that the dose and volume of the solution should always be uniform. This is, of course, obviously impossible, since the body-weight varies among individuals. It must be remembered that volume and concentration of the solution introduce variable factors. The amount of glycogen stored in the body also influences the utilization of sugar, together with the amount of muscular work being done during the experiment. The effects of hunger — hunger glycosuria — we may leave out of consideration. It is said that the external temperature influences the utilization of sugars, a high temperature favoring an alimentary glycosuria. Individual peculiarities also probably play an important rôle.

Raimann reports a raised assimilation limit in idiocy, mania, paranoia, in epileptics and in alcoholics after the disappearance of the mental disturbance. Strauss reports no especial tendency to alimentary glycosuria in hysteria and epilepsy. Sigmund[10] noted a transitory glycosuria in 7.4 per cent. of epileptics.

It may be noted that in the course of several thousand examinations of the urine of epileptics, glycosuria has been an extremely rare finding at the Craig Colony.

In the work reported in the present paper, test doses were administered in the form of a sirup, flavored with lemon extract to appeal to the palate. The gross weight of dextrose used was disregarded as we found that the commercial dextrose used did not titrate at its expected strength; therefore each individual batch of sirup was carefully titrated against carefully standardized Fehling's solution. Sirups ranged in strength from about 45 gm. to 70 gm. to 100 c.c.

In our work the dose was administered about 10 or 11 a. m.; these patients have breakfast between 6:30 and 7 o'clock, so that the stomach should have been fairly empty at this time. We found that many patients vomited at dinner-time, and when giving larger doses, it was decidedly a wise procedure to refuse dinner to the experimental persons. At first urines were collected at frequent intervals, but we found that all practical purposes were served by samples taken at 2 and 4 p. m., respectively. Control urines taken before the doses were given in no case showed glycosuria.

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[10] Sigmund: Handbuch der Biochemie, iv, part ii, 153.

In examining the urine samples, Fehling's test was used entirely. Dextrose is apparently present in normal urine to a very small amount, but is not recognizable by Fehling's solution, so that it does not enter into our work. Simon states that the ordinary tests, including Fehling's, are worthless for detecting the occurrence of alimentary glycosuria, and directs the use of the fermentation test as the determining test. He assigns no reason for this. If, however, we consider the utilization of dextrose and the qualities of the tests employed, we shall have a much better idea of the value of the several testing methods.

Dextrose is completely utilized and is excreted as water and carbon dioxid. This change does not immediately take place, however, some dextrose being deposited as glycogen and some being only partially oxidized, forming glycuronic acid. The latter product may appear in the urine if it is formed in excessive quantities, and it is also a fair assumption that if the amount of dextrose offered for assimilation is excessive, more of the glycuronic acid will be formed and excreted. The free acid is not fermentable by yeast, but readily reduces Fehling's solution without boiling. Glycuronic acid does not appear free in the urine, but in combination with phenol, indoxyl, or skatoxyl, and in this form it is only very slowly reducing for Fehling's solution and then only at boiling temperatures. Dextrose is easily reduced by Fehling's solution without boiling and is fermentable by yeast. The use of the fermentation test would exclude glycuronic acid, but the same may be done by performing Fehling's test properly, that is, without boiling. The latter test has the advantage, however, that by the later thorough boiling of it, the presence of glycuronic acid may be recognized. There are other bodies which may give small reductions in this part of the test; when overdoses of dextrose are being given, we may regard these as negligible.

In our work we have used the Fehling test, both with and without boiling, in the belief that the dextrose or an excess of glycuronic acid were equally significant. Cases in which the tests were marked positive with a question-mark indicate reductions which have occurred after boiling. These reactions were characterized by the production of yellow or orange precipitates, and never has a dirty-green cloudiness, which so often occurs, been accepted as a reduction, even though reduction may occur in such a test if allowed to stand a sufficient length of time.

The proportion of positive results among the higher ratios is evident (Table 4), but even so, the number whose tolerance was passed is very much smaller than the number of those who gave negative results.

Of the two positives indicated as occurring at ratios between 2 and 3 gm. per kilogram, it must be remarked that in one, there was apparently a transitory renal disturbance, as indicated by an excess of cylindroids, casts and leukocytes. This patient afterward tolerated a much larger dose, at a time when the urine was clear. In the other instance, the test was very doubtfully positive, and is the only test on that patient.

Theoretically, and judging from the results of Cushing's work, administration of gland extract should cause a lowering of the assimilation limit, and doses of sugar given during administration of gland substance should produce glycosuria at a much lower rate of "grams per kilogram" than without the drug. In our experiments on this end of the subject, we have considered doses of 2.5 gm. per kilogram as representing the normal limit. The amount of gland substance required to produce glycosuria with this dose should represent the deficiency of the patient's own gland. Table 5 gives the results of our work in this direction. It will be noted that in the last three groups, the amount of dextrose used is considerably increased. This was done as we found that the lower ratios were not giving results, and rather than increase the dose of the expensive gland preparation we preferred first to increase the sugar and try and obtain positives in this way.

TABLE 4.—Size of Doses of Dextrose, in Terms of Grams per Kilogram of Body-Weight, and Result

Grams Per Kg.	Total Cases	Positive Result
2 to 3	13	2
3 to 4	26	0
4 to 5	13	1
5 to 6	14	5
6 to 7	10	2
7 to 8.15.................	8	2
	— 84	— 12

It will be seen that the tolerance is not lowered in the majority of these cases. The ratio of positives is not much different from that seen in the control series for maximum tolerance, namely, one-seventh of the cases in each group show positive; but a comparison of the amounts of sugar used will show that the positives obtained with coincident gland administration were, on the whole, obtained with much lower amounts of sugar. Probably the small doses of gland substance are too small, and we see that the positives in the groups taking from 20 to 30 grains three times a day, seventeen in all, show four positives, or nearly one-quarter. These cases were given sugar between ratios of 3.08 and 4.95. Comparing with the previous table, we see that between 3 and 5 there were thirty-nine cases with only one positive among them.

TABLE 5.—Results of Gland Administration

Grains T.I.D.	Experiments	Ratio Used	Result
3	9	2.35 : 2.85	0
6	7	2.69 : 3.09	1 positive
10	37	2.53 : 4.8 ; One case, 1.62	5 positive
15	1	3.7	0
20	12	3.08 : 3.48	3 positive
30	5	4.0 : 4.95	1 positive
	71		10 positive

Some of the particular cases may be cited from the table:

Babcock, positive (?) at 7.06 per kilogram; positive with 10 grains of gland, ratio, 2.76 negative with 30 grains of gland, ratio, 4.48.

Neville, positive 2.3 grams — urine shows casts, etc.; negative at 2.2, 2.7, 3.9; positive with 6 grains t. i. d., and 2.82 gm.; negative with 10 grains t. i. d., and 3.68 gm.

Wilde, negative with 5.9 gm. sugar per kilogram; with 20 grains gland t. i. d., gave doubtful negative with 3.39 gm. sugar.

Klotz, negative with 6 gm.; positive with 10 grains t. i. d., and 3.1 gm. sugar; negative with 10 grains t. i. d., and 3.1 gm. sugar; negative with 30 grains t. i. d., and 3.7 gm. sugar.

McDermott, negative with 8.15 gm. sugar; positive with 10 grains gland t. i. d., and 2.72 gm. sugar; negative with 30 grains gland t. i. d., and 4.75 gm. sugar.

McArthur gave negative with 10 grains gland t. i. d., and 2.76 and 4.13 gm. sugar; positive with 10 grains gland t. i. d., and 2.66 gm. sugar.

It will be seen from these instances that the reaction to this set of experiments has not been uniform in all cases. It is evident that some variable factor either in the method or in the patient is causing an apparent variation of the assimilation limit for dextrose. It will require considerably more work with these patients to determine the basis of these variations.

It seems reasonable, however, to conclude, even from the work at this stage, that there is a definite and marked elevation of the assimilation limit in these patients. Whether or not this has any relation to the activity of the pituitary gland is not shown clearly enough at present to permit any statement.

THE SPECIFIC RÔLE OF FOODS IN RELATION TO THE COMPOSITION OF THE URINE *

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INTRODUCTION

The influence of individual foods on the composition of the urine has been studied from several points of view. Many of the relations have been satisfactorily determined.

It is a matter of common knowledge that the urine of herbivorous animals is alkaline to litmus, while that of the carnivora is acid in reaction. Human urine, likewise, is normally acid. The alkaline urine of herbivora has been supposed to result from the vegetable foods on which they live. Similarly, the acid reaction of the carnivorous urine was referred to the meat diet of these animals. What is present in these foods that may be held responsible for the differences in urinary acidity? The excess of base-forming elements in vegetables and of acid-forming elements in meats at once suggests an answer. It was not until Sherman and Gettler[1] made more complete ash analyses of a large number of foods that the importance of this point was sufficiently impressed. Their studies showed that the vegetables and fruits, on burning, leave an ash preponderating in the basic elements, sodium, potassium, calcium and magnesium. The cereals, meat and fish foods give an acid-reacting ash because of a predominance of the acid-forming elements. The important fact must not be overlooked that the ash of plant foods may be either basic or acidic. Throughout this investigation, the analyses just referred to have been kept in mind (Table 1).

Another factor which may directly affect the acid formation within the body is the protein intake. Its content of sulphur and phosphorus leads to the production of acids, which require that a certain amount of base be supplied for neutralization. On the other hand, Robertson[2] has shown that the serum proteins are efficient in neutralizing acids. He found these body proteins to be about one-fifth as capable of neutralizing acids as are the phosphates and bicarbonates.

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* Submitted for publication, May 2, 1914.

* From the Sheffield Laboratory of Physiological Chemistry, Yale University.

* Reported before the American Society of Biological Chemists, Philadelphia, Dec. 31, 1913.

1. Sherman and Gettler: Jour. Biol. Chem., 1912, xi, 323.
2. Robertson: Jour. Biol. Chem., 1910, vii, 351.

TABLE 1.—Ash Analyses of Sherman and Gettler: Excess of Acid-Forming or Base-Forming Elements[1]

Articles of Food	Excess Acid or Base in Terms of Normal Solutions			
	Per 100 Gm.		Per 100 Calories	
	Acid c.c.	Base c.c.	Acid c.c.	Base c.c.
Almonds	.....	12.38	.....	1.86
Apples	.....	3.76	.....	5.98
Asparagus	.....	0.81	.....	3.65
Bananas	.....	5.56	.....	5.62
Beans, dried	.....	23.87	.....	6.92
Beans, lima, dried	.....	41.65	.....	12.08
Beets	.....	10.86	.....	23.57
Cabbage	.....	4.34	.....	13.76
Carrots	.....	10.82	.....	23.91
Cauliflower	.....	5.33	.....	17.48
Celery	.....	7.78	.....	42.17
Cherry juice	.....	4.40		
Chestnuts	.....	7.42	.....	3.19
Corn, sweet, dried	5.95	.....	1.77	
Crackers	7.81	.....	1.95	
Currants, dried	.....	5.97	.....	1.85
Eel	9.89	.....		
Eggs	11.10	.....	7.55	
Egg white	5.24	.....	9.52	
Egg yolk	26.69	.....	7.08	
Fish, haddock	16.07	.....		
Fish, pike	11.81	.....		
Lemons	.....	5.45	.....	12.32
Lettuce	.....	7.37	.....	38.69
Meat, beef, lean	13.91	.....	12.10	
Meat, chicken	17.01	.....		
Meat, frog	10.36	.....		
Meat, pork, lean	11.87	.....		
Meat, rabbit	14.80	.....		
Meat, veal	13.52	.....		
Meat, venison	15.83	.....		
Milk, cow's	.....	2.37	.....	3.44
Muskmelon	.....	7.47	.....	18.82
Oatmeal	12.93	.....	3.23	
Oranges	.....	5.61	.....	10.94
Peaches	.....	5.04	.....	12.20
Peanuts	3.9	.....	0.70	
Peas, dried	.....	7.07	.....	1.98
Potatoes	.....	7.19	.....	8.63
Prunes	.....	24.40	.....	8.05
Radishes	.....	2.87	.....	9.79
Raisins	.....	23.68	.....	6.87
Raspberry juice	.....	4.91		
Rice	8.1	.....	3.35	
Turnips	.....	2.68	.....	6.86
Wheat, entire	9.66	.....	3.25	
Wheat, flour	11.61	.....	2.70	

Urinary acidity is dependent on the composition of the blood. This fluid maintains a very constant, slightly alkaline reaction, as has been observed by many investigators. How is its uniform reaction maintained? The most important factor in bringing this about is the presence of salts of carbonic and phosphoric acids. Systems consisting of H_2CO_3, $NaHCO_3$, NaH_2PO_4 and Na_2HPO_4, such as are found in the blood, have great powers for maintaining neutrality (Henderson).[3] Thus, in a phosphate solution, relatively large amounts of base may be neutralized, with very small changes in reaction. The acids of the blood leave the body by two channels, the carbonic going chiefly by way of the lungs. The remaining acids are excreted by the kidneys. The latter organs, therefore, have a very important rôle in relation to the body acids and consequently in the preservation of body neutrality. The case of phosphoric acid serves to emphasize the importance of the kidney's function along these lines. This acid exists in the blood largely as Na_2HPO_4, while it is found in the urine chiefly as NaH_2PO_4. Thus, in passing through the kidneys, a great share of the base has been retained and is again ready for further combination with acid. Since phosphoric acid is one of the chief acids of the urine, the value of this phenomenon is very significant. Of the acids produced in metabolism, only sulphuric removes all of the base required for neutralization. It becomes apparent that the introduction of bases into the body offers a ready supply for the neutralization of acids formed in metabolism. Depending on the quantities introduced, this may significantly change the composition of the blood and, as a consequence, the make-up of the urine. The study of urinary acidity, therefore, becomes highly important.

The true acidity of the urine is due to an excess of H^+ ions in the excretion. In terms of physical chemistry, an excess of H^+ ions in solution causes acidity, while a surplus of $\overline{OH}$ ions produces alkalinity. Whenever there is an equal number of H^+ and $\overline{OH}$ ions present, the solution becomes neutral.

H^+ ion concentration is expressed as gram of ionized H per thousand grams of water. A neutral solution has a H^+ of 1 times 10^{-7} or 0.000,000,1 gm. per liter. As a matter of convenience in recording, the H^+ is often expressed by using the logarithmic notation of Sörensen. Thus, at neutrality the H^+ is 7.00. As the H^+ increases, this value decreases, and with an increasing $\overline{OH}$ the value increases. In our protocols, the H^+ is expressed in this fashion.

Not until Henderson and Palmer[4] developed their indicator method for determining the H^+ of the urine, have we possessed a ready and accurate method for the estimation of true urinary acidity. Formerly, the H^+ was found by the laborious gas-chain method. Because of the complications involved, this procedure was not suitable for general use. The indicator method makes use of the well-known fact that the color of an indicator varies with changes in H^+.

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[3] Henderson: Am. Jour. Physiol., 1908, xxi, 427.

[4] Henderson and Palmer: Jour. Biol. Chem., 1913, xiii, 393.

Thirteen solutions of known H^+ are prepared, the concentration of the most acid being 4.70 and that of the most alkaline 9.27. Corresponding volumes of the standards and of the urines under investigation are diluted to a definite volume, and the same amount of indicator added to each. The color of the urine sample is then matched with the standards and its position in the series located. Since equal volumes of the standards and of the urines are used, any two having the same color and intensity must have the same H^+. The method is rapid and is especially applicable to experiments in which the subjects are maintained on a uniform diet, for one is then able to forecast in what range of H^+ the urines will fall. The necessity of trying different ranges and indicators is therefore eliminated.

Using this method, Henderson and Palmer[4] reported that the H^+ of urines of normal individuals living on a mixed (?) diet ranges from 4.82 to 7.45, with an average of 6.03. Very recently (1914), these authors[5] have published further observations on 122 specimens, the average H^+ of which was 5.94. The mean value of those previously recorded and of that found in their latest studies is 5.98. These authors believe that under general conditions of activity and diet the H^+ is equal to 6 ± 0.1. They also stated that under pathological conditions the tendency is for the H^+ to become greater. This was especially observed in cardiorenal cases, in which the average value of 5.33 was obtained.

At this point, it may be desirable to consider the relation of H^+ to the urinary acidity as determined by the titration methods which are commonly used. Titration, with a base, does not measure the true acidity. It merely gives the excess of acid substances, as shown by some indicator. That titration methods are not without objection is easily gleaned from the admirable discussion by Henderson:[6]

> Without exception such procedures consist in titrating to the reaction which chances to be a convenient "end-point" of a particular indicator. But such a point is in itself quite without necessary physiological significance, for there is in the nature of things no relation between any physiological constant or process and the reaction at which phenolphthalein, or litmus, or alizarine, or any other indicator changes color sharply. Each of these concentrations of ionized hydrogen is dependent in an indeterminate way on the chemical constitution of the indicator, and upon nothing else. Any other relationship is

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[5] Henderson and Palmer: Jour. Biol. Chem., 1914, xvii, 305.

[6] Henderson: Jour. Biol. Chem., 1911, ix, 403.

purely accidental. Nor is there even in chemical theory that prescription of a particular indicator which may always be found in the case of the titration of any simple solution. This fact is amply attested by the difficulties which have been encountered in devising a suitable titration method to estimate the excess of acid over base in urine. Among the urinary constituents are some whose "neutralization" is incomplete at any possible end-point. In solutions which are just pink with phenolphthalein ammonia is free in measureable amount, and the proteins and amino-acids are combined with a considerable and uncertain quantity of base. Between such alkalinity and the acidity of the methyl orange end-point the phosphates and many other substances are combined with varying amounts of base. Finally at a more acid reaction hippuric acid, lactic acid, and other substances are present partly as salts and partly free, while, on the other hand, the proteins and their derivatives are in part united with acid.

Phenolphthalein is the indicator most generally used, because it marks most nearly the end of a number of chemical reactions.

To show that H^+ and titratable acidity are very different, one needs only to consider solutions of the common acids. Normal solutions of hydrochloric and acetic acids will require the same amount of base for neutralization, but the H^+ of the hydrochloric is much greater than that of the acetic. This is due to the fact that the molecules of hydrochloric are dissociated to a greater extent. Acids which have a small ionization constant are termed weak acids. The urine resembles the weak acids, such as acetic, but its H^+ is less and its capacity for combining with bases greater. This is due to the presence of weak acids and their salts, especially NaH_2PO_4 and Na_2HPO_4.

Organic salts of the alkalies are widely employed as therapeutic agents, those most commonly used being the bicarbonate and citrate of sodium. Many of the organic acids are oxidized to carbonates in the body. The carbonic acid is given off through the lungs, the base remaining to combine with acids produced by the body. It has long been known that the urine can be rendered alkaline to litmus by the administration of alkalies. The extent of this reduction in H^+ has been determined by Henderson and Palmer.[7] On giving alkalies, such as the citrate and bicarbonate of sodium, they were able to obtain a H^+ of 8.70, which corresponds to the alkalinity of a solution of 0.01 N Na_2HPO_4. Hanzlik and Collins[8] observed a similar effect during their studies on the conversion of hexamethylenetetramin into formaldehyd in the urine.

The belief that ammonia is produced within the body to neutralize the acid products of metabolism is generally accepted. Supporting this conclusion is the often-observed fact that the output of ammonia in the urine is decreased when alkalies are given. That is, the alkalies

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7. Henderson and Palmer: Jour. Biol. Chem., 1913, xiv, 81.

8. Hanzlik, P. J., and Collins, R. J.: Hexamethylenamin: The Liberation of Formaldehyd and the Antiseptic Efficiency under Different Chemical and Biological Conditions, The Archives Int. Med., 1913, xii, 578.

TABLE 2.—

Date 1913	Volume c.c.	Specific Gravity	Acidity N/10, c.c.	[H⁺]	Ammonia N, gm.
SUBJECT B					
April		1.013	216	7.48	0.234
14	1,500	1.017	288	7.14	0.323
15	1,075	1.012	267	7.38	0.314
16	1,450	1.020	271	6.90	0.309
17	890	1.015	266	7.14	0.316
18	1,145	1.016	284	7.38	0.292
19	1,060				
SUBJECT W					
April					
14	890	1.022	253	7.43	0.224
15	1,070	1.021	300	7.14	0.283
16	955	1.022	264	7.38	0.287
17	820	1.024	253	7.14	0.236
18	930	1.022	286	7.38	0.268
19	880	1.024	264	7.38	0.286

TABLE 3.—Basal—

SUBJECT B					
April					
7	1,150	1.020	221	7.48	0.234
8	1,020	1.023	196	7.74	0.221
9	1,135	1.020	200	7.74	0.231
10	1,260	1.019	202	7.74	0.212
11	1,250	1.018	190	7.74	0.270
12	1,350	1.016	216	7.74	0.243
13	1,150	1.019	212	7.74	0.248
SUBJECT W					
April					
7	925	1.026	204	7.48	0.289
8	775	1.027	210	7.43	0.279
9	1,100	1.024	180	7.74	0.211
10	1,315	1.020	195	7.74	0.221
11	1,220	1.019	190	7.74	0.264
12	1,345	1.019	194	7.74	0.242
13	1,285	1.021	206	7.74	0.201

TABLE 4.—

SUBJECT B					
May					
26	1,210	1.015	305	6.70	0.334
27	1,330	1.013	229	6.80	0.279
28	1,300	1.014	208	7.14	0.218
29	1,360	1.015	250	7.48	0.245
30	1,040	1.018	225	7.48	0.237
SUBJECT W					
May					
26	835	1.024	294	6.70	0.310
27	975	1.021	230	6.80	0.293
28	920	1.023	265	7.38	0.298
29	980	1.023	261	7.48	0.226
30	860	1.025	248	7.48	0.206

—Basal Diet

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
9.40	0.422	0.781	+0.359	Basal diet. Graham crackers, 100 gm. Dairy-butter, 25 gm. Whole milk, 400 c.c. per meal. For supper one soft-boiled egg and one raw apple extra.
10.25	0.459	0.432	—0.027	
10.53	0.438	0.718	+0.280	
10.27	0.490	0.443	—0.047	
10.59	0.440	0.539	+0.099	
10.35	0.447	0.435	+0.012	
8.87	0.377	0.604	+0.227	Basal diet. Graham crackers, 100 gm. Dairy-butter, 25 gm. Peanut-butter, 10 gm. Whole milk, 400 c.c. each meal. For supper one soft-boiled egg and one raw apple extra.
11.06	0.430	0.585	+0.155	
10.89	0.466	0.669	+0.203	
11.02	0.433	0.478	+0.045	
12.00	0.425	0.492	+0.067	
11.40	0.438	0.588	+0.150	

—Diet with Potatoes

10.42	0.503	1.13	+0.627	Basal diet with 750 gm. baked potatoes a day.
10.04	0.494	1.09	+0.596	
10.20	0.508	1.27	+0.762	
10.81	0.480	1.39	+0.710	
11.18	0.485	1.44	+0.955	
10.64	0.499	1.54	+1.04	
10.29	0.492	1.03	+0.538	
11.60	0.459	0.885	+0.426	Basal diet and 600 gm. baked potatoes a day.
9.81	0.406	0.564	+0.158	
11.15	0.472	1.230	+0.758	
10.82	0.440	1.670	+1.230	
10.41	0.444	1.360	+0.916	
11.12	0.520	1.730	+1.210	
10.10	0.469	1.580	+1.110	

—Potato Ash

10.47	0.479	0.418	—0.061	Potato ash corresponding to 750 gm. of baked potatoes begun, May 28.
9.27	0.412	0.614	+0.202	
9.20	0.453	0.973	+0.520	
10.08	0.472	1.080	+0.608	
9.22	0.408	0.745	+0.337	
11.10	0.476	0.448	—0.028	Potato ash corresponding to 600 gm. of baked potatoes, beginning on the third day.
10.20	0.450	0.654	+0.204	
10.07	0.493	0.533	+0.040	
11.21	0.434	0.720	+0.286	
9.71	0.372	0.690	+0.318	

supply a not insignificant amount of the bases required for neutralization, and the ammonia formation is consequently reduced. Janney[9] has reported cases in which doses of 15 gm. $NaHCO_3$ produced a decrease in the output of ammonia to one-third of its normal value. Using larger amounts, he was able to practically suppress the ammonia excretion.

These effects on H^+ and ammonia excretion, which are referable to the intake of bases, are very striking. They give an idea as to the cause of the varying reaction of the urine. In general, foods are burned by the body much as in the crucible, an inorganic residue being the product in both instances. The ash is alkaline in the case of vegetables and fruits and acid when meats and cereals are consumed. Theoretically, we are led to expect that the eating of potatoes, which yield a strongly alkaline ash, will have the same effect on H^+ and ammonia excretion as does the ingestion of comparable amounts of sodium bicarbonate or other suitable bases. Likewise, one would assume that meat-eating should cause the production of a more acid urine. An indication that such may be the case is found in the metabolism experiment conducted by Sherman and Gettler.[1] They reported that the eating of potatoes decreased, while the ingestion of rice, which yields an acid-reacting ash, increased the output of ammonia.

It is common knowledge that experiments conducted *in vivo* and *in vitro* very often differ in their results. In view of the practical importance of this problem, we have made an extended study of the influence of food intake on the acidity of the urine and of the related phenomena.

The tendency for the formation of uric acid sediments in certain urines is often observed. Other urines do not exhibit this phenomenon. Those specimens which produce sediments are always acid in reaction and generally of small volume. Hindhede[10] has reported in numerous papers that urines obtained after eating vegetables of different kinds not only show no tendency to the formation of sediments but have a heightened ability to dissolve uric acid. Potato urines are especially striking in this respect. Other vegetables and fruits which Hindhede has shown to have this same effect on the urine are bananas, carrots, melons, strawberries, tomatoes and rhubarb. Milk urines also exhibit the same phenomenon. On the other hand, urines from a bread or a meat diet not only fail to dissolve more uric acid but deposit some of that already in solution, showing that they are supersaturated. Hindhede followed the titratable acidity in these cases and found that there was apparently a relationship between this and the capacity for dissolving uric acid. Those specimens having a low titratable acidity were able to bring added uric acid into solution, while those of high acidity suffered precipitation. This investigator believed that the volume of the urine also had some connection with this phenomenon but was inclined to place more emphasis on the other factor. It seemed that work along this line should be confirmed and extended. Consequently, the solvent power of urine for uric acid has been made a part of our study.

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[9] Janney: Ztschr. f. physiol. Chem., 1912, lxxvi, 99.

[10] Hindhede: Skandin. Arch. f. Physiol., 1912, xxvi, 384; ibid., 1912, xxvi, 87; Ztschr. f. diätet. u. physik. Therap., 1913, xvii, 592.

METHODS

H^+ ION CONCENTRATION

The new indicator method of Henderson and Palmer,[4] described above, was used in the determination of true acidity.

SOLUBILITY OF URIC ACID

The procedure used in the estimation of the solubility of uric acid in urine is as follows:

About 125 c.c. of urine are placed in a glass-stoppered bottle. To this is added about 0.5 gm. of pure uric acid. The bottles are then closed and sealed by dipping the necks in melted paraffin. They are placed on a rotating machine and revolved for two hours at room temperature, care being taken not to rotate so rapidly as to produce foam. The urines are filtered and 200 c.c. of the filtrate used for the determination of uric acid. From this result the value for the twenty-four-hour specimen is calculated. This is the total amount of uric acid that the urine is capable of dissolving. We have called this the "total solvent power." The difference between this and the uric acid excreted represents the capacity of the urine for dissolving uric acid, beyond the amount already present. This is recorded as "residual solvent power." A positive sign indicates solution and a negative sign shows precipitation. All determinations of uric acid were made by the Folin-Shaffer method.

This procedure is not open to certain objections which may be offered to Hindhede's[11] method for the determination of the extent of solution. His procedure is outlined below:

Two hundred c.c. of urine are boiled for a few minutes, filtered and again made up to volume with water. Fifty c.c. are transferred to a dried and weighed flask, which contains 100 mg. of uric acid. The flask is placed in a thermostat at 37 C. (98.6 F.) for an hour, and shaken several times during the interval. The contents are filtered,

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[11] Hindhede: Skandin. Arch. f. Physiol., 1912, xxvi, 384.

TABLE 5.—Alkali, Rice, Wheat—

Date 1913	Volume c.c.	Specific Gravity	Acidity N/10, c.c.	[H⁺]	Ammonia N, gm.
SUBJECT B					
April					
20	1,210	1.015	198	7.48	0.174
21	1,320	1.015	132	8.00	0.127
22	1,350	1.017	130	8.35	0.113
23	1,155	1.015	166	7.48	0.166
24	1,085	1.018	203	7.38	0.176
26	1,025	1.020	267	6.80	0.197
27	1,045	1.018	297	6.90	0.251
28	1,165	1.021	350	6.80	0.280
29	1,175	1.021	413	6.70	0.395
30	1,150	1.022	378	6.70	0.373
1	1,540	1.018	482	6.50	0.610
SUBJECT W					
April					
20	940	1.024	203	7.48	0.237
21	870	1.025	129	8.00	0.122
22	975	1.028	109	8.35	0.047
23	900	1.025	205	7.38	0.206
24	925	1.023	266	7.14	0.278
26	1,195	1.020	272	6.80	0.244
27	860	1.024	295	6.90	0.299

TABLE 6.—Oranges—

SUBJECT B					
May					
10	1,550	1.014	235	7.48	0.391
11	1,460	1.015	292	7.48	0.333
12	1,200	1.016	293	7.14	0.288
13	1,290	1.015	289	7.38	0.279
14	1,090	1.018	306	7.38	0.275
15	1,130	1.020	249	7.43	0.190
16	1,080	1.020	259	7.38	0.233
17	1,410	1.015	226	7.48	0.246
18	1,150	1.019	290	7.48	0.249
SUBJECT W					
May					
10	1,030	1.024	206	7.43	0.309
11	900	1.026	230	7.43	0.292
12	900	1.025	238	7.38	0.167
13	980	1.023	235	7.43	0.235
14	905	1.026	235	7.48	0.168
15	850	1.028	248	7.43	0.168
16	800	1.028	253	7.43	0.134
17	1,010	1.026	222	7.48	0.206
18	780	1.027	253	7.43	0.178

—Bread, and a Mixed Diet

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
9.65	0.413	1.17	+0.757	5 gm. $NaHCO_3$
10.07	0.398	1.50	+1.102	10 gm. $NaHCO_3$
9.93	0.429	2.62	+2.191	15 gm. $NaHCO_3$
9.44	0.422	1.32	+0.898	Rice, 210 gm.
9.19	0.427	0.790	+0.363	Rice, 210 gm.
9.97	0.463	0.523	+0.060	Rice, 210 gm.
10.04	0.465	0.445	—0.020	Rice, 210 gm.
12.54	0.578	0.530	—0.038	Whole-wheat bread, 450 gm.
13.25	0.617	0.469	—0.148	
12.33	0.635	0.508	—0.147	
15.09	0.755	0.639	—0.116	Mixed diet.
12.00	0.444	1.110	+0.666	5 gm. $NaHCO_3$
11.10	0.444	1.180	+0.726	10 gm. $NaHCO_3$
10.68	0.449	1.430	+0.981	15 gm. $NaHCO_3$
10.75	0.493	0.921	+0.528	Rice, 210 gm.
10.12	0.420	0.521	+0.101	Rice, 210 gm.
11.04	0.456	0.676	+0.220	Rice, 210 gm.
11.10	0.429	0.410	—0.019	Rice, 210 gm.

—and Raisins

10.06	0.443	0.922	+0.479	Oranges, 800 gm.
10.57	0.495	1.050	+0.555	Oranges, 800 gm.
10.67	0.482	0.692	+0.210	Oranges, 800 gm.
10.46	0.553	0.887	+0.334	Oranges, 1,300 gm.
9.08	0.531	0.670	+0.139	Raisins, 300 gm.
8.62	0.467	0.995	+0.528	Raisins, 300 gm.
8.23	0.450	0.875	+0.420	Raisins, 300 gm.
8.04	0.437	1.310	+0.873	Raisins, 300 gm.
8.57	0.442	0.764	+0.322	Raisins, 300 gm.
10.80	0.510	1.060	+0.550	Oranges, 800 gm.
10.41	0.478	1.060	+0.562	Oranges, 800 gm.
10.41	0.438	0.790	+0.352	Oranges, 800 gm.
10.38	0.443	0.961	+0.518	Oranges, 1,300 gm.
9.24	0.448	0.880	+0.432	Raisins, 300 gm.
8.80	0.425	0.828	+0.403	Raisins, 300 gm.
8.48	0.395	0.832	+0.437	Raisins, 300 gm.
9.01	0.450	1.230	+0.780	Raisins, 300 gm.
7.45	0.383	0.692	+0.309	Raisins, 300 gm.

in the thermostat, through a dried and weighed filter. Both flask and filter are removed from the thermostat and washed thoroughly with cold water. The flask and filter are dried and weighed. The difference between the first and last weighings indicates the amount that has been dissolved. The method is tedious and not without liability to errors. The excess of uric acid might offer opportunity for absorption of urine. The large positive values for the solubility of uric acid obtained by this investigator, however, leave no doubt as to the occurrence of the phenomenon.

OTHER METHODS

The methods for the estimation of titratable acidity and ammonia were those of Folin. Total nitrogen was determined by the Kjeldahl method.

PLAN OF EXPERIMENTS

The subjects were given a uniform diet consisting of 100 gm. graham crackers, 25 gm. dairy-butter and 400 c.c. whole milk at each of the three daily meals. For the evening meal, one soft-boiled egg and a raw apple were added. The latter articles serve a very useful purpose in relieving the relative blandness of the diet. In some cases, when more nitrogen was desired, 10 or 20 gm. of peanut-butter was given at each meal. This ration is ample for protein and energy requirements. No restriction was placed on the amount of water or salt consumed. When data for this basal diet had been established, the effect of the addition of the food in question was studied. Liberal amounts were given, so as to leave no doubt in regard to the interpretation of the results. Whenever possible, the food was eaten in the natural, uncooked state. With such a plan it is not difficult to determine the influence of any food on the composition of the urine. The body quickly reacts to the change, and the new equilibria will generally have been established by the second day. The effect of each food was studied for several days. Somewhat less than a week is sufficient to obtain conclusive data.

Twenty-four-hour specimens of urine were collected, toluene being used as a preservative. The experimental day ended just before breakfast. Analyses were always made as soon as possible after the samples were completed.

The subjects were healthy young men who recognized the need of care in the collection of specimens. No restrictions were placed on them beyond that of diet.

EXPERIMENTAL DATA

SERIES I

Basal Diet (Table 2).—The choice of the diet just described was very fortunate, since it yielded urines which were practically neutral in reaction, thus making more evident any changes produced by the experimental foods. The average H+ for the period of six days was 7.31 for Subject W and 7.24 for Subject B, giving a mean value of 7.28. This corresponds very well with the figure (7.34) obtained by Howe and Hawk,[12] using the gas-chain method under similar experimental conditions. It is indeed interesting to note the close agreement in results obtained by the indicator and gas-chain procedures. The values for ammonia N were very constant in both cases, varying with Subject W from 0.224 to 0.287 gm. and with Subject B from 0.234 to 0.323 gm. per day. The uric acid excretion showed typical endogenous figures, the diet being practically purin-free. It will be observed that in these urines the value for the "total solvent power" for uric acid was not greatly different from the amount excreted; that is, in some cases solution took place and in others precipitation occurred. Since these urines were practically neutral, this fact led us to believe that the extent of the solution of uric acid in urine is dependent on H+. As will appear later, further data supported this conclusion.

Potatoes (Table 3).—The addition of baked potatoes to the basal ration produced a decrease in H+, as was anticipated because of the excess of basic elements in the ash (Table 1). The value in both cases was 7.74. Ammonia N remained about the same as with the basal diet in the case of Subject W, but was decreased slightly in that of the other subject. The most interesting change brought about was in the "total solvent power" for uric acid, when in every instance, solution of added uric acid took place, the maximum value being 1.23 gm. and the minimum 0.158 gm. It is interesting to note that an increased capacity for the solution of uric acid was associated with a decrease in H+.

Administration of Alkali, Rice, Wheat Bread, and a Mixed Diet (Table 5).—Varying amounts of sodium bicarbonate were introduced, ranging from 5 gm. on the first day to 10 gm. on the second and 15 gm. on the third day. It was taken by mouth in three equal portions, before meals. In both subjects, there was a gradually decreasing H+, accompanied by a decreasing ammonia output and by an increasing solvent power for uric acid. On the third day, Subject W excreted only 0.047 gm. of ammonia N, in contrast with his normal output of about 0.2 gm. This clearly illustrates the ability of ingested base to curb ammonia production. The lowest H+ (8.35) came on the third day, when the bicarbonate intake was the greatest.

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[12] Howe and Hawk: Proc. Amer. Soc. Biol. Chem., Jour. Biol. Chem., 1914, xvii, 48.

TABLE 7.—New—

Date 1913	Volume c.c.	Specific Gravity	Acidity N/10, c.c.	$[H^+]$	Ammonia N, gm.
SUBJECT B					
July					
9	1,320	1.015	370	5.70	0.317
10	1,040	1.020	458	5.70	0.412
12	1,475	1.013	560	5.85	0.434
13	620	1.028	518	5.30	0.550
14	940	1.017	466	5.30	0.608
SUBJECT I					
July					
9	975	1.020	429	5.70	0.445
10	1,715	1.013	460	5.70	0.504
12	1,250	1.014	475	5.30	0.495
13	1,310	1.012	482	5.30	0.573
14	1,590	1.011	483	5.50	0.553

TABLE 8.—

SUBJECT B					
July					
15	1,285	1.015	570	5.30	0.602
16	1,700	1.012	578	5.30	0.705
17	710	1.025	540	5.30	0.630
19	1,520	1.016	578	4.80	0.729
SUBJECT I					
July					
15	1,410	1.013	524	5.30	0.559
16	1,610	1.011	537	5.30	0.522
17	1,715	1.010	548	5.30	0.597
19	1,325	1.015	551	4.80	0.635

TABLE 9.—

SUBJECT B					
August					
23	1,880	1.011	376	5.70	0.452
24	915	1.023	476	5.30	0.560
25	830	1.025	604	5.30	0.708
26	985	1.021	614	5.10	0.568
27	1,040	1.020	600	5.30	0.588
28	930	1.023	554	5.30	0.447
SUBJECT I					
August					
23	1,120	1.015	363	5.50	0.417
24	1,290	1.016	469	5.30	0.558
25	1,165	1.016	519	4.90	0.643
26	845	1.020	524	5.10	0.538
27	870	1.019	490	5.10	0.564
28	940	1.017	481	5.30	0.463

—Basal Diet

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
7.68	0.472	.....	....	Basal diet. Whole-wheat crackers, 100 gm.; whole milk, 400 c.c.; dairy-butter, 25 gm. each meal. One soft-boiled egg and one raw apple at supper.
8.46	0.510	0.373	—0.137	
8.85	0.495	0.470	—0.025	
8.33	0.508	0.121	—0.387	
9.42	0.445	0.262	—0.183	
9.00	0.336	.....	....	Basal diet. Whole-wheat crackers, 75 gm.; whole milk, 400 c.c.; dairy-butter, 25 gm. each meal. One soft-boiled egg and one raw apple at supper.
8.95	0.340	0.070	—0.270	
8.85	0.302	0.093	—0.209	
8.95	0.288	0.069	—0.219	
9.45	0.320	0.077	—0.243	

—Prunes

9.43	0.453	0.105	—0.352	Prunes 330 gm. a day.
9.49	0.434	0.115	—0.319	
7.77	0.396	0.138	—0.258	Prunes, 330 gm. a day.
7.21	0.417	0.113	—0.304	Prunes, 550 gm. a day.
8.90	0.268	0.068	—0.200	Prunes, 240 gm. a day.
9.28	0.238	0.071	—0.167	
9.06	0.318	0.084	—0.234	Prunes, 240 gm. a day.
7.67	0.269	0.071	—0.198	Prunes, 330 gm. a day.

—Plums

7.56	0.427	0.348	—0.079	Plums, 200 gm. each meal.
8.00	0.418	0.224	—0.194	
9.61	0.392	0.165	—0.227	
10.29	0.410	0.161	—0.249	
10.62	0.437	0.199	—0.238	
10.00	0.418	0.157	—0.261	
6.80	0.335	0.155	—0.180	Plums, 150 gm. each meal.
7.81	0.305	0.093	—0.212	
7.97	0.305	0.100	—0.205	
7.72	0.297	0.112	—0.185	
8.36	0.298	0.136	—0.162	
7.69	0.295	0.130	—0.165	

Rice, being a food with a predominance of acid-forming elements, was introduced immediately after the bicarbonate period, in order to magnify the effects of acid production. A gradual rise in H^+ and ammonia and a decreasing ability for uric acid solution were produced. The effects of this acid food became apparent only slowly, on account of the increased store of alkali caused by the previous bicarbonate ingestion. The urines did not show an acid reaction until the end of three days. The ammonia output increased and smaller amounts of added uric acid were dissolved until, on the fourth day, precipitation occurred, showing the urines to be supersaturated.

Subject B continued the experiment for three days on a diet of whole-wheat bread and a final day on a mixed diet. This change brought an increase in H^+ and ammonia and a uniform, negative solution (precipitation) of uric acid. Reference to Table 5 shows how negative values for uric acid solubility accompany an acid urine. This experiment illustrates well the readiness with which the body responds to changes in diet, in order to maintain the usual neutrality.

Potato Ash (Table 4).—The purpose of this experiment was to determine whether or not the effects produced by the potato diet were directly due to the excess of basic, inorganic elements present.

The potatoes were peeled and ashed. This procedure left a gray residue, which was strongly alkaline and partially soluble in water. Since it was impossible to take this product directly, it was given in capsules and even in this form great discomfort was experienced. Soon after its ingestion, a burning sensation developed in the stomach and persisted for some time thereafter. Quantities of ash corresponding to the weights of baked potatoes eaten in the former experiment were employed, the ash being given in three equal portions.

The data show a close similarity in both cases. The effects produced by the ash were less marked than those caused by equivalent amounts of baked potatoes, but this was probably incident to the relative insolubility of the ash. At no time was such a low H^+ reached; but, after the first day, the uric acid solubility showed uniformly positive values, which were greater than those obtained with the basal diet. Therefore, it seems just to conclude that the effects produced by the ingestion of potatoes are referable to the preponderance of basic elements present.

Oranges and Raisins (Table 6).—In order to secure the greatest possible influence of these foods, extra quantities were eaten between meals. Care was taken to obtain chemically untreated raisins. Very similar results were obtained with both fruits, namely, a low H^+ and ammonia output and a uniformly high capacity for the solution of uric acid. When compared with the basal diet, this experiment shows effects comparable to those found when sodium bicarbonate was taken, and justifies the opinion that these results are due to the basic content of the foods in question.

SERIES II

Opportunity was afforded, during the summer vacation of 1913, for experimental studies in the physiological laboratory of the Battle Creek Sanitarium. I take pleasure in expressing my appreciation of the many kindnesses extended by Dr. J. H. Kellogg and other members of the staff. I am also deeply indebted to Miss Ada Hunt, who very efficiently supervised the preparation of the various diets.

New Basal Diet (Table 7).—This diet was similar to the first, with the exception that whole-wheat crackers were substituted for the graham crackers. This change brought forth an unexpected result, in that a very acid urine was excreted by both subjects. An H^+ of 5.30 was obtained in the two cases, which value is greater than that of the normal human urine, as observed by Henderson and Palmer[5] (1914). Accompanying this were high values for ammonia excretion, typical figures being 0.553 gm. in the case of Subject I and 0.550 gm. in that of Subject B. All of the urines showed a "negative solubility" of uric acid which was more pronounced with Subject I, in whose case in all specimens over 0.2 gm. of this substance was precipitated. The low endogenous uric acid excretion of Subject I is referable to his small weight.

Prunes (Table 8).—As reference to Table 1 shows that prunes are characterized by their great preponderance in basic elements, it seemed highly probable that they would be an ideal food to overcome the highly acid basal diet. Our surprise was great, therefore, when an increased H^+ and ammonia excretion were observed in both cases. On the last day, when the greatest amount of dried prunes was taken, the H^+ was 4.80 for both subjects. Ammonia N values of 0.635 and 0.729 gm., in the urines of Subjects I and B, respectively, prevailed in contrast with the normal output of about 0.56 gm. The large "negative solubility" of uric acid continued throughout this period. This unforeseen result was made clear only by further work with plums, cranberries and benzoic acid.

Plums (Table 9).—The interesting point in connection with fresh plums was to determine whether or not their influence would be similar to that of prunes (dried plums). It is impossible to eat as much of the fresh plums as of the dried prunes, owing to their difference in moisture content. Marked urinary changes were not observed. While no very definite increase in H^+ and ammonia was found, there was no decrease; and on the third day Subject I showed

TABLE 10.—

Date 1913	Volume c.c.	Specific Gravity	Acidity N/10, c.c.	[H⁺]	Ammonia N, gm.
SUBJECT B					
July					
20	1,095	1.020	466	5.30	0.513
21	1,920	1.012	338	6.30	0.323
22	1,370	1.017	285	7.38	0.255
23	1,535	1.015	298	7.14	0.240
24	1,525	1.017	250	7.38	0.220
SUBJECT I					
July					
20	1,950	1.010	437	5.70	0.492
21	2,285	1.009	343	6.50	0.356
22	1,700	1.012	265	6.90	0.245
23	1,680	1.012	215	7.38	0.181
24	1,960	1.012	227	7.38	0.188

TABLE 11.—

SUBJECT B					
July					
26	1,205	1.014	347	5.70	0.406
27	725	1.022	473	5.30	0.479
28	810	1.025	395	5.70	0.311
29	775	1.025	388	5.70	0.307
30	705	1.028	457	5.50	0.389
31	635	1.029	485	5.30	0.447
SUBJECT I					
July					
26	940	1.018	369	6.00	0.376
27	950	1.016	433	4.90	0.502
28	780	1.022	418	4.90	0.412
29	755	1.023	390	5.30	0.417
30	890	1.021	434	5.50	0.417
31	770	1.022	462	5.30	0.426

TABLE 12.—VEGETARIAN—

SUBJECT B					
August					
2	1,160	1.013	316	5.70	0.445
3	935	1.014	348	5.30	0.618
4	555	1.029	369	5.30	0.380
5	585	1.028	332	5.50	0.412
6	495	1.031	426	5.30	0.366
7	1,080	1.016	363	5.70	0.402
SUBJECT I					
August					
2	1,015	1.016	313	5.70	0.317
3	810	1.020	211	6.90	0.282
4	650	1.023	231	6.00	0.277
5	640	1.022	279	5.70	0.369
6	770	1.018	311	5.50	0.416
7	885	1.016	280	5.70	0.362

—Cantaloup

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
7.93	0.477	0.316	—0.161	Cantaloup, 260 gm. each meal (one melon).
9.32	0.557	0.598	+0.041	
9.38	0.603	0.426	—0.177	
9.87	0.625	0.600	—0.025	
9.48	0.611	0.690	+0.079	
8.07	0.374	0.095	—0.279	Cantaloup, 295 gm. each meal (one melon).
8.50	0.336	0.449	+0.113	
8.37	0.410	0.594	+0.184	
8.26	0.499	0.766	+0.267	
8.47	0.462	1.006	+0.544	

—Dasheen Flour.

7.74	0.455	0.288	—0.167	Dasheen flour, 80 gm. daily.
7.78	0.481	0.144	—0.337	
8.61	0.540	0.303	—0.237	
8.78	0.562	0.332	—0.230	
8.68	0.535	0.168	—0.367	
8.68	0.494	0.126	—0.368	
8.19	0.349	0.288	—0.061	Dasheen flour, 60 gm. daily (sanitarium).
7.44	0.282	0.055	—0.217	
7.68	0.340	0.076	—0.264	
7.90	0.338	0.143	—0.195	
8.03	0.308	0.163	—0.145	
8.13	0.322	0.102	—0.220	

—Diet (Sanitarium)

6.58	0.551	0.448	—0.103	Vegetarian diet (sanitarium).
5.88	0.432	0.142	—0.290	
6.70	0.764	0.117	—0.647	
6.67	0.508	0.246	—0.262	
6.28	0.600	0.120	—0.480	
6.74	0.620	0.296	—0.324	
6.08	0.396	0.276	—0.120	Vegetarian diet (sanitarium).
5.59	0.460	0.450	—0.010	
5.37	0.392	0.323	—0.069	
5.02	0.380	0.145	—0.235	
5.14	0.388	0.112	—0.276	
5.21	0.425	0.201	—0.224	

a high value of 4.90 for H^+. Constantly high values for ammonia N were found in all cases.

Cantaloup (Table 10).—The outcome of this experiment was one of the most satisfactory that we obtained in the entire series. The cantaloup period began on the day following that in which the highly acid conditions, due to the prunes, were met. The effect of this food is well shown in the case of Subject I, when starting with a H^+ of 4.80 on the last prune-diet day there was a steady decrease until this value became 7.38 on the fourth cantaloup-diet day. Similarly, the ammonia N dropped from 0.635 to 0.181 gm. On the second day, positive values for solution of uric acid appeared and continued throughout the period. Subject B responded in essentially the same manner. The values for H^+ changed from 4.80 to 7.38 on the third day, and the ammonia data show a steady decrease from 0.729 to 0.220 gm. An increased excretion of uric acid, amounting to as much as 0.15 gm., appeared in both cases. The cause for this is uncertain. It is possible that uric acid was held back during the highly acid prune period and was then washed out in the alkaline urines resulting from the cantaloup. Indeed, inspection of the values for uric acid excretion during the prune period shows that these were somewhat below the normal.

Dasheen Flour (Table 11).—The dasheen is a plant which is quite similar to the potato (Young[13]). It is a member of the family *Araceae* and of the genus *Colocasia* and has been introduced into this country by the United States government, from the island of Trinidad and the West Indies. The corms and tubers of these plants are similar to the potato but their content of starch and protein is about one-half higher than in the potato, owing to their smaller percentage of water. Since the fresh tubers were not in season, a dasheen flour was used, which was cooked in the form of a gruel and as muffins. It was impossible to eat more than 80 gm. per day, so that no definite effects were produced.

Vegetarian Diet (*Sanitarium*) (Table 12).—Recognizing the fact that vegetables are characterized by a preponderance of basic elements, we investigated the varied lactovegetarian (sanitarium) diet with the expectation of obtaining a low acid output. The two subjects did not respond as anticipated, although the urines of many normal persons living on the same diet had low acidity. These data will be discussed later. Subject I gave a maximum value of 5.50 for H^+ and a minimum figure of 6.90. His ammonia N output ranged from 0.277 to 0.416 gm., while the uric acid solubilities were in every instance negative. Subject B showed a variation in ammonia N from 0.366 to 0.618 gm. A heavy sediment of uric acid was present on the fifth day, which was hardly to be expected with such a diet. An interesting disclosure was the low output of nitrogen—5.4 gm. daily in the case of Subject I and 6.5 gm. in the other. Both were at liberty to eat all they wished, so these figures may perhaps be taken as fairly representing the normal output of nitrogen of persons living on such dietaries.

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[13] Young: Bur. Plant. Ind., Circ. 127, U. S. Dept. Agric., 1913.

Tomatoes (Table 13).—Because of the high water content of tomatoes, it is not easy to eat large enough amounts to cause any profound change. Nevertheless, significant departures from the normal allowed the conclusion that the tomato does cause a decrease in H^+ and ammonia excretion. In the case of Subject I, the H^+ varied from 5.70 to 5.85 and the ammonia N from 0.236 to 0.334 gm., the latter being uniformly lower than the basal diet figures. Subject B on three days gave a H^+ of 6.00 and his ammonia N varied between 0.246 and 0.448 gm. Negative values for solubility of uric acid were constantly found.

Celery (Table 14).—The celery was eaten both in the natural state and in the form of a soup, part of the allowance of milk being used in the preparation of this. No significant changes were observed.

Dates (Table 15).—Considerable annoyance was experienced during this study because of the gastro-intestinal fermentation induced by the dates. This disturbance was so severe that Subject I was compelled to end the experiment after the second day, while Subject B continued two days longer. Reference to the table shows no effects which may be related to this food.

Nut Food (Table 16).—This food, a preparation of peanuts, intended to supplant meats in vegetarian diets, is a concentrated food and when taken in undue amounts is rather difficult to assimilate. In these studies, this product was eaten broiled. Low values for ammonia N, without a significant decrease in H^+, were obtained in both cases. The cause of this anomaly remains unexplained.

Cauliflower (Table 17).—This food was thoroughly cooked in a pressure autoclave. It became very distasteful after the third day, so that the experiment was discontinued. In both instances, the first effects seemed to be a decreased output of acid and ammonia, but these were followed by steadily increasing amounts. Subject I, for example, showed at the start a H^+ of 6.00 and ended with the value 5.50. The corresponding figures for ammonia N were 0.237 and 0.490 gm.

Sodium Bicarbonate (Table 18).—Typical results of the administration of alkali, as such, were again found. Ten gm. of sodium

TABLE 13.—

Date 1913	Volume c.c.	Specific Gravity	Acidity N/10, c.c.	[H⁺]	Ammonia N, gm.
SUBJECT B					
August					
9	705	1.022	226	5.70	0.246
10	660	1.027	451	5.70	0.301
11	2,075	1.010	474	6.00	0.448
12	935	1.022	399	5.70	0.281
13	1,030	1.020	346	6.00	0.247
14	870	1.023	381	6.00	0.272
SUBJECT I					
August					
9	625	1.022	275	5.70	0.236
10	890	1.020	427	5.70	0.246
11	1,420	1.012	426	5.70	0.307
12	840	1.020	360	5.85	0.252
13	840	1.020	363	5.70	0.322
14	870	1.020	378	5.70	0.334

TABLE 14.—

SUBJECT B					
August					
16	920	1.020	368	5.70	0.326
17	730	1.026	473	5.30	0.368
18	875	1.028	514	5.50	0.368
19	830	1.030	534	5.30	0.388
20	690	1.029	466	5.30	0.389
21	810	1.029	466	5.30	0.379
SUBJECT I					
August					
16	715	1.023	412	5.30	0.378
17	780	1.024	452	5.30	0.450
18	775	1.026	412	5.50	0.381
19	830	1.024	441	5.30	0.468
20	630	1.024	406	5.30	0.443
21	700	1.024	423	5.30	0.420

TABLE 15.—

SUBJECT B					
August					
30	1,250	1.017	400	5.30	0.405
31	780	1.026	455	5.30	0.403
1	740	1.027	468	5.30	0.338
2	750	1.026	435	5.50	0.301
SUBJECT I					
August					
30	1,210	1.019	397	5.30	0.480
31	1,065	1.016	417	5.30	0.428

—Tomatoes

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
6.97	0.534	0.454	—0.080	Tomatoes, 300 gm. each meal.
8.67	0.597	0.170	—0.427	
10.81	0.644	0.457	—0.187	
9.69	0.575	0.489	—0.086	
10.41	0.588	0.562	—0.026	
9.94	0.572	0.455	—0.117	
5.23	0.312	0.245	—0.067	Tomatoes, 200 gm. each meal.
7.64	0.336	0.206	—0.130	
8.14	0.335	0.276	—0.059	
7.56	0.366	0.315	—0.051	
8.52	0.338	0.232	—0.106	
8.47	0.298	0.218	—0.080	

—Celery

9.39	0.541	0.451	—0.090	Celery, 150 gm. each meal.
8.73	0.528	0.192	—0.336	
10.47	0.585	0.260	—0.325	
10.80	0.594	0.171	—0.423	
9.52	0.475	0.118	—0.357	
10.02	0.525	0.188	—0.337	
8.06	0.302	0.159	—0.143	Celery, 100 gm. each meal.
7.80	0.376	0.169	—0.207	
8.17	0.328	0.167	—0.161	
8.62	0.310	0.087	—0.223	
7.48	0.346	0.113	—0.223	
8.43	0.377	0.108	—0.269	

—Dates

8.27	0.577	0.313	—0.264	Dates, 150 gm. each meal.
7.68	0.503	0.201	—0.302	
7.86	0.453	0.169	—0.284	
8.20	0.447	0.200	—0.227	
7.91	0.397	0.139	—0.258	Dates, 100 gm. each meal.
6.78	0.272	0.087	—0.183	

bicarbonate daily gave a H⁺ of 7.38 in the case of Subject I and of 6.70 in that of Subject B, while 15 gm. caused a further reduction to 8.00 in both cases. Another very low excretion of ammonia N was observed in Subject I on the second day, when only 0.072 gm. was excreted. Uric acid showed practically no tendency to precipitate; indeed, in the case of Subject I the values for the excretion and "total solvent power" were identical. Circumstances made it impossible to carry out these determinations on the second experimental day.

TABLE 16.—

Date 1913	Volume c.c.	Specific Gravity	Acidity N/10, c.c.	$[H^+]$	N, gm. Ammonia
SUBJECT B					
September					
9	1,130	1.022	427	5.70	0.244
10	950	1.027	502	5.70	0.285
11	1,030	1.025	494	5.70	0.241
SUBJECT I					
September					
9	890	1.024	392	5.70	0.235
10	745	1.024	465	5.30	0.277
11	950	1.023	460	5.50	0.239

TABLE 17.—

SUBJECT B					
September					
13	1,830	1.016	380	6.00	0.362
14	1,020	1.026	563	5.50	0.515
15	1,450	1.018	609	5.50	0.697
SUBJECT I					
September					
13	1,040	1.020	316	6.00	0.237
14	1,300	1.017	354	6.00	0.328
15	1,020	1.020	465	5.50	0.490

TABLE 18.—SODIUM—

SUBJECT B					
September					
16	1,270	1.015	335	6.70	0.305
17	1,950	1.012	226	8.00	0.140
SUBJECT I					
September					
16	820	1.019	256	7.38	0.256
17	1,200	1.017	154	8.00	0.072

SERIES III

Basal Diet (Table 19).—The original basal diet of graham crackers, whole milk, dairy-butter and peanut-butter was used for the remaining experiments in New Haven. Here again is noticed the approximate neutrality of urines resulting from this diet. The average H^+ of all the urines of the four subjects, during a period of five days, was 7.10, or a slight alkalinity. Corresponding to this value were comparatively low figures for ammonia N, Subjects H and M excreting greater amounts than did the two others, on account of their higher nitrogen intake. Uric acid solubility was determined only in the urines of Subject W. The previously observed phenomenon of occasional precipitation was found to hold for these practically neutral urines.

—Nut Food

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
11.53 12.38 13.63	0.579 0.593 0.598	0.405 0.206 0.321	—0.174 —0.387 —0.277	Nut food, 100 gm. each meal.
9.64 9.97 11.52	0.356 0.330 0.416	0.271 0.111 0.198	—0.085 —0.219 —0.218	Nut food, 100 gm. each meal.
—Cauliflower				
11.10 12.34 13.80	0.651 0.744 0.720	0.596 0.191 0.227	—0.055 —0.553 —0.493	Cauliflower, 150 gm. each meal.
8.23 9.63 9.50	0.456 0.483 0.470	0.360 0.386 0.117	—0.096 —0.097 —0.353	Cauliflower, 100 gm. each meal.
—Bicarbonate				
11.32	0.535	0.509	—0.026	$NaHCO_3$, 10 gm.
.....	.....	.....	....	$NaHCO_3$, 15 gm.
7.98	0.363	0.363	+—0.000	$NaHCO_3$, 10 gm.
.....	.....	.....	....	$NaHCO_3$, 15 gm.

Bananas (Table 20).—The influence of this food is clearly exhibited in the case of Subject W, whose urines gave a uniform value of

7.43 for H⁺ throughout, as against an average of 7.19 on the basal diet. The ammonia N dropped from an original value of over 0.2 gm. to an average of about 0.15 gm. daily. The most striking observation was in the change of solubility of uric acid from practically zero to a positive value of 0.4 gm. dissolved. The same effects were observed in the case of Subject M.

Apples (Table 21).—Two large raw apples, besides the usual one at supper, were eaten with meals, the skins not being discarded. A decrease in H⁺ was found in both instances. The average H⁺ of Subject B on the basal diet (6.86) became 7.38 under the influence of apples, while the corresponding values for Subject H were 7.24 and 7.43. A small but irregular decrease in ammonia N was observed in both cases.

Benzoic Acid (Table 22).—It will be recalled that plums and prunes caused an increased acid excretion, which was contrary to the theoretical deductions from the ash analyses. The most plausible explanation is that these fruits contain some substance which is noncombustible in the body and is excreted in the form of a relatively strong acid. Benzoic acid, which is found in prunes to the extent of 0.05 per cent. (Radin[14]), is excreted as hippuric acid.

To determine the effect of benzoic acid on urinary acidity, 1 gm. in a capsule was taken before each meal. At no time were any ill effects noticed by any of the three subjects. The results of the acid ingestion are well illustrated by Subject W, who showed an increase in H⁺, which reached 5.30 and was at no time less than 6.30. Paralleling this was an average daily output of ammonia N of 0.392 gm., as against 0.242 gm. with the basal diet. Likewise, negative solution (precipitation) of uric acid obtained in all the urines. Comparable effects were shown by Subjects B and M.

An increased acid-production due to the ingestion of benzoic acid is not hard to explain, since it is well known that from it the kidneys synthesize hippuric acid, the strongest of the urinary acids. Judging from previous experience, the augmented ammonia formation which accompanied this increased acid elimination was to be expected. These results are not in harmony with those obtained with pigs by M'Collum and Hoagland,[5] who reported absence of an increase in ammonia when benzoic acid was fed. Their tables, however, show a noticeable increase in this factor.

Further evidence that benzoic acid ingestion increases the acidity is revealed in the different studies made on urinary antiseptics, such as hexamethylenetetramin, which requires an acid reaction before formaldehyd can be liberated from it. Walker[16] and Jordan[17] have demonstrated that benzoic acid and ammonium benzoate are efficient in bringing about the requisite acid condition.

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[14] Radin: Unpublished data from this laboratory, 1914.

[15] M'Collum and Hoagland: Jour. Biol. Chem., 1913, xvi, 321.

Cranberries (Table 23).—These berries have long been the classic example of a natural food containing benzoic acid. Their content of this acid is 0.06 per cent. (Radin).[14] In view of the interesting disclosures resulting from the preceding experiment, we deemed it worth while to include this food in our study. The berries were taken in the familiar form as a sauce. No discomfort was observed even when the original amount of 300 gm. of sauce daily was increased to 600 gm. on the sixth day. Large quantities of fecal matter were passed, which probably explains the diminished excretion of urinary N encountered in the latter days of the experiment.

The data obtained from Subject W may be taken as truly representative of the conditions prevailing in all four subjects. On the last day of greatest cranberry intake, a H^+ of 5.70 was found and the corresponding value for ammonia N was 0.346 gm., both being marked increases over those obtained with the basal diet. Negative values for solubility of uric acid were exhibited by all the urines. The unanimity of results obtaining for these four subjects justifies the belief that the acid production following cranberry-eating is caused by their content of benzoic acid.

The H^+ of the Urines of Thirty Vegetarians (Table 24).—Unusual opportunity was at hand for obtaining urines of persons who had lived on a vegetarian diet for varying lengths of time, some having been exclusive vegetarians for twenty-five years. The subjects were physicians, nurses, bath-room attendants and helpers about the Battle Creek Sanitarium. All were required to turn in a written report of their diet and were carefully instructed regarding the collection of samples. The twenty-four-hour specimens were preserved with toluene. The thirty samples were from as many persons, both men and women. Reference to the table shows a range in H^+ from 5.30 to 7.48, with an average value of 6.64, which is somewhat less than that of 5.98 reported by Henderson and Palmer[5] for normal human urine. Doubtless, their observations were made on individuals living on an ordinary mixed diet. The diet lists of those persons who produced alkaline urines showed that they were eating heartily of potatoes and cantaloup, both of which are efficient in decreasing acid formation. It is reasonable to expect that a vegetarian diet, with its preponderance in basic elements, will cause the production of a less acid urine than will a mixed diet in which the meats, with their surplus of acid-

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[16] Walker: Brit. Med. Jour., 1913, ii, 643.
[17] Jordan: Brit. Med. Jour., 1913, ii. 654.

TABLE 19.—

Date 1913	Volume c.c.	Specific Gravity	Acidity N/10, c.c.	[H⁺]	Ammonia N, gm.
SUBJECT B					
October					
19	1,090	1.016	305	6.70	0.282
20	1,290	1.015	325	6.90	0.248
21	1,310	1.013	346	6.90	0.251
22	1,460	1.014	374	6.90	0.245
23	1,200	1.016	338	6.90	0.216
SUBJECT H					
October					
19	1,010	1.016	258	6.90	0.303
20	1,200	1.018	273	7.38	0.382
21	2,040	1.011	302	7.38	0.539
22	1,050	1.019	323	7.38	0.346
23	1,455	1.015	352	7.14	0.412
SUBJECT M					
October					
19	1,355	1.014	311	6.90	0.521
20	2,180	1.011	305	7.43	0.314
21	1,060	1.020	369	7.38	0.465
22	940	1.022	363	6.90	0.502
23	1,060	1.019	377	6.90	0.413
SUBJECT W					
October					
19	945	1.019	340	6.90	0.284
20	1,030	1.020	359	7.38	0.204
21	1,350	1.017	367	7.38	0.268
22	1,170	1.019	393	7.14	0.239
23	1,000	1.021	408	7.14	0.216

TABLE 20.—

SUBJECT M					
October					
31	1,600	1.015	301	7.48	0.288
1	1,230	1.021	271	7.43	0.332
2	1,460	1.015	257	7.48	0.289
3	1,190	1.017	257	7.43	0.315
4	1,400	1.015	185	7.43	0.269
SUBJECT W					
October					
31	1,450	1.015	296	7.43	0.148
1	1,250	1.018	240	7.43	0.157
2	1,330	1.016	293	7.43	0.144
3	1,260	1.018	282	7.43	0.121
4	1,360	1.016	223	7.43	0.139

—Basal Diet

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
9.82	.....	.....	....	Basal diet. 100 gm. Graham crackers; whole milk, 1 pint; dairy-butter, 25 gm., each meal. One soft-boiled egg and one raw apple extra for supper.
10.22	.....	.....	....	
10.29	.....	.....	....	
10.12	.....	.....	....	
10.65	.....	.....	....	
9.71	.....	.....	....	Basal diet. Graham crackers, 100 gm.; whole milk, 1 pint; dairy-butter, 25 gm.; peanut-butter, 20 gm. These were taken each meal. At supper one soft-boiled egg and one raw apple.
12.20	.....	.....	....	
13.22	.....	.....	....	
10.83	.....	.....	....	
13.31	.....	.....	....	
11.58	.....	.....	....	Basal diet. Graham crackers, 100 gm.; whole milk, 1 pint; dairy-butter, 25 gm., and peanut-butter, 20 gm. each meal. One soft-boiled egg and one raw apple extra for supper.
13.34	.....	.....	....	
11.58	.....	.....	....	
12.85	.....	.....	....	
12.24	.....	.....	....	
10.54	.....	.....	....	Basal diet. Graham crackers, 100 gm.; whole milk, 1 pint; dairy-butter, 25 gm., and peanut-butter, 10 gm. each meal. One soft-boiled egg and one raw apple extra at supper.
11.00	0.381	0.369	—0.012	
11.78	0.391	0.610	+0.219	
11.68	0.400	0.112	—0.288	
11.85	0.393	0.382	—0.011	

—Bananas

12.34	.....	.....	....	Bananas, about 310 gm. each meal.
10.12	.....	.....	....	
11.53	.....	.....	....	
9.71	.....	.....	....	
10.34	.....	.....	....	
9.88	0.393	0.873	+0.480	Bananas, about 310 gm. each meal.
10.60	0.398	0.841	+0.443	
10.27	0.370	0.752	+0.382	
10.62	0.378	0.779	+0.401	
10.13	0.410	0.973	+0.563	

forming elements, constitutes a significant part. Nevertheless, the fact must not be forgotten that a vegetarian diet, if it is composed largely of the cereals, may be just as acidic or even more than a mixed diet.

TABLE 21.—

Date 1913	Volume c.c.	Specific Gravity	Acidity N/10, c.c.	$[H^+]$	Ammonia N, gm.
SUBJECT B					
October					
31	1,720	1.010	407	6.90	0.320
1	2,110	1.008	279	7.38	0.190
2	1,760	1.010	359	7.38	0.306
3	1,810	1.010	304	7.38	0.239
4	2,110	1.009	295	7.43	0.190
SUBJECT H					
October					
31	2,300	1.008	239	7.38	0.372
1	1,870	1.011	292	7.43	0.337
2	1,510	1.014	278	7.43	0.389
3	1,750	1.012	266	7.43	0.357
4	1,670	1.012	294	7.43	0.350

TABLE 22.—

SUBJECT B					
October					
24	1,620	1.011	392	6.15	0.292
26	1,640	1.013	410	6.15	0.374
27	1,600	1.013	443	6.00	0.422
28	1,620	1.013	434	6.00	0.408
29	1,340	1.015	468	5.70	0.418
SUBJECT M					
October					
24	1,250	1.018	325	6.70	0.428
26	1,660	1.013	382	6.70	0.678
27	1,850	1.013	437	6.70	0.600
28	1,175	1.020	470	6.30	0.628
29	1,370	1.017	420	6.70	0.608
SUBJECT W					
October					
24	930	1.022	458	6.30	0.302
26	1,480	1.014	435	6.00	0.400
27	1,060	1.021	518	5.70	0.438
28	1,320	1.017	470	5.70	0.388
29	1,090	1.020	538	5.30	0.432

H^+ ion Concentration and the Carbon-Dioxid Tension of the Alveolar Air (Table 25).—The determination of the tension of alveolar CO_2 has recently come into prominence as a diagnostic measure in cases of acidosis. It is particularly useful in advanced cases of

diabetes. In these, the tension of CO_2 is always lower than normal. This lowered value is attributed to a diminished alkalinity of the blood, which decreases the CO_2 in the blood and, therefore, the tension of this gas in the pulmonary alveoli.

—Apples

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
8.37	.....	.....	....	Two apples extra each meal. About 2,300 gm. daily.
7.67	.....	.....	....	
9.24	.....	.....	....	
8.53	.....	.....	....	
8.85	.....	.....	....	
9.26	.....	.....	....	Two apples extra with each meal, about 2,300 gm. daily.
10.99	.....	.....	....	
10.33	.....	.....	....	
11.66	.....	.....	....	
10.63	.....	.....	....	

—Benzoic Acid

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
10.84	.....	.....	....	Benzoic acid, 1 gm., in a capsule before each meal.
9.94	.....	.....	....	
9.76	.....	.....	....	
10.46	.....	.....	....	
10.59	.....	.....	....	
13.06	.....	.....	....	Benzoic acid, 1 gm., before each meal.
12.44	.....	.....	....	
12.38	.....	.....	....	
12.34	.....	.....	....	
13.00	.....	.....	....	
11.38	0.385	0.216	—0.169	Benzoic acid, 1 gm., before each meal.
10.83	0.367	0.256	—0.111	
10.97	0.385	0.156	—0.229	
11.32	0.402	0.222	—0.180	
10.49	0.350	0.063	—0.287	

It seemed desirable to discover the behavior of the H^+ of the urine in connection with the alveolar CO_2 tension and to establish a relationship, if possible. The Haldane[18] method was used in the determin-

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18. Haldane and Priestly: Jour. Physiol., 1905, xxxii, 225.

ations of the alveolar CO_2 tension.[19] The samples were always taken at the same hour, before breakfast. The subjects sat in an easy position for at least five minutes before the air was collected. Triplicate estimations of the air taken at the end of a forced expiration, following normal inspiration, were always made. Unfortunately, during this series of trials only one diet (cantaloup) caused the production of an alkaline urine. Reference to Table 25 shows that, during the prune period, Subject B had a CO_2 tension of 34 mm. with a H^+ of 5.30. Under the influence of the cantaloups, the CO_2 tension increased to a maximum of 38.2 mm. and the H^+ decreased to 7.38. On returning to the acid diet, a drop in CO_2 tension was observed. At no time during the remainder of the experiment were these high values for CO_2 tension of the cantaloup diet again reached. The ingestion of 15 gm. sodium bicarbonate, daily, produced a tension of CO_2 of 36.7 mm. and a H^+ of 8.00. The other subject responded in a similar manner, although the values obtained were not so uniform. The breathing of this subject was very shallow, which made the collection of the samples of air difficult. It will be observed that Subject B had a lower CO_2 tension than normal. This is probably explained by his habitually deep respirations, which allowed of more complete ventilation.

The change in CO_2 tension appears more quickly than does that in the H^+ of the urine. Thus, on the last prune-diet day Subject B gave a CO_2 tension of 34.09 mm., which became 37.0 mm. on the first day on cantaloups. The corresponding variations in H^+ were only slight, from 4.80 to 5.30. It was to be expected that the blood would respond more quickly, because of its great power of maintaining a constant reaction.

Hasselbalch[20] (1912) has reported similar changes brought about by variations in diet. The values for CO_2 tension obtained with one of this author's subjects were as follows; meat diet 37.8 mm., mixed diet 38.3 mm., vegetarian diet 43.3 mm., and alkaline diet ($NaHCO_3$) 42.1 mm. Hasselbalch says:

> A diet which produces an acid urine causes a low carbon dioxid tension of the blood; a diet that produces a less acid or an alkaline urine causes at the same time a high carbon dioxid tension of the blood.[21]

H. Straub[22] has stated that these changes in the CO_2 tension of alveolar aid produced by diet are only temporary. He is of the opinion that the CO_2 tension soon returns to normal, and may even be normal in cases of diabetes, provided the acidosis is not too great.

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[19] These estimations were made by Dr. Paul Roth of the Battle Creek Sanitarium.

[20] Hasselbalch: Biochem. Ztschr., 1912, xlvi, 403.

[21] Eine Diät, die einen sauren Harn verursacht, bewirkt eine niedrige CO_2-Spannung des Blutes; eine Diät, die einen weniger sauren oder alkalischen Harn verursacht, bewirkt gleichzeitig eine hohe CO_2-Spannung.

[22] Straub, H.: Deutsch. Arch. f. klin. Med., 1913, cix, 223.

The H^+ of blood at different pressures of CO_2 has been studied by Hasselbalch and Lundsgaard[23] (1911). They found that, at a pressure of about 40 mm. CO_2, a change of 1 mm. of CO_2 pressure made a difference of only 0.0065 times 10^{-7} in the H^+ of the blood. It has been shown by Campbell, Douglas, Haldane and Hobson[24] (1913) that a difference of about 0.8 mm. in the normal tension of alveolar CO_2 of 40 mm. gives an increase of 50 per cent. in the alveolar ventilation. This corresponds to a change in H^+ of only 0.005 times 10^{-7}. From this it becomes evident that the respiratory center, during normal life, responds to very minute changes in the H^+ of the blood. Since the CO_2 tension of alveolar air remains remarkably constant, it is plain that the chief regulation of the H^+ of the blood takes place in other organs besides the lungs. All evidence points to the kidneys as exerting the greatest rôle in the maintenance of this constancy in reaction.

Higgins[25] (1914) has very recently reported observations which indicate that some other factor besides the H^+ of the blood is acting on the respiratory center. He finds that the alveolar CO_2 tension is markedly influenced by posture, being higher when the subject is standing than sitting and higher when sitting than lying. It was also noted that beefsteak and sugar ingestion produced an increased CO_2 tension, lasting as long as the food was being absorbed. Coffee drinking caused a decrease in the CO_2 tension of alveolar air. From this author's experience, there is reason to believe that a high CO_2 tension of alveolar air is associated with vasodilatation, and a low alveolar CO_2 tension with vasoconstriction.

DISCUSSION

When the preceding results are carefully observed, it readily becomes apparent that urinary acidity, the output of ammonia and the solvent power for uric acid can be materially changed by appropriate variations in diet. It has been my purpose to show that these changes are referable to the character of the ash yielded by the several foods, so far as this represents likewise the end-result of the oxidation of the nutrients in the organism. In this respect, the foods or diets may be said to possess a potential acidity or alkalinity. The possibility of the presence of certain organic substances which are excreted as acids must be taken into account; for example, benzoic acid. In general, the results obtained have been those which were expected. Thus, foods which yield an alkaline ash decrease the urinary acidity, while those yielding an acid-reacting residue increase this factor. In order to make more clear the relations existing between the different urinary factors, they will be considered from various points of view.

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[23] Hasselbalch and Lundsgaard: Biochem. Ztschr., 1911, xxxviii, 77.
[24] Campbell, Douglas, Haldane and Hobson: Jour. Physiol., 1913, xlvi, 301.
[25] Higgins: Am. Jour. Physiol., 1914, xxxiv, 114.

TABLE 23.—

Date 1913	Volume c.c.	Specific Gravity	Acidity N/10, c.c.	$[H^+]$	Ammonia N, gm.
SUBJECT B					
November					
6	1,000	1.018	413	6.15	0.264
7	1,255	1.014	391	6.30	0.219
8	1,460	1.012	356	6.30	0.280
9	1,500	1.013	354	6.70	0.288
10	1,600	1.014	423	6.30	0.269
11	1,090	1.016	432	5.85	0.380
12	1,230	1.015	488	5.70	0.391
SUBJECT H					
November					
6	865	1.021	291	6.70	0.462
7	830	1.021	462	6.30	0.537
8	1,890	1.013	492	6.70	0.601
9	965	1.019	355	6.70	0.394
10	960	1.021	346	6.70	0.444
11	1,060	1.019	373	6.70	0.534
12	1,630	1.012	359	6.70	0.528
SUBJECT M					
November					
6	1,060	1.018	403	6.90	0.522
7	1,215	1.017	379	6.90	0.423
9	1,630	1.011	372	6.90	0.431
10	1,520	1.014	322	6.90	0.383
11	1,510	1.015	387	6.90	0.734
12	825	1.024	419	6.30	0.610
SUBJECT W					
November					
6	985	1.021	422	6.50	0.237
7	915	1.021	403	6.50	0.198
8	1,030	1.019	470	6.00	0.303
9	880	1.022	359	6.70	0.253
10	1,030	1.020	446	6.00	0.266
11	1,280	1.016	451	6.00	0.330
12	960	.....	442	5.70	0.346

THE RELATION OF H^+ TO TITRATABLE ACIDITY

Determinations of the excess of acid over basic radicals were made along with estimations of the true acidity or the H^+ ion concentration. Our results agree with those very recently reported by Henderson and Palmer,[5] who reached the conclusion that titratable acidity is a function of the H⁺. Thus, they found that the average values of A (titratable acidity) steadily diminished from 320 c.c. N/10 NaOH with a H^+ of 5.4 to 224 c.c. with a H^+ of 6.6. It is their belief that in the more acid specimens the variations in acidity are dependent on variations in the manner of the treatment of constant amounts of phosphoric acid by the regulatory mechanism, and results simply in a change in the proportion of acid to alkaline phosphate in the urine. The following hypotheses are offered by them: (*a*) With a constancy in the excretion of phosphoric acid, the H^+ will vary with A according to the mass law equilibrium of phosphoric acid. (*b*) With a con-

—Cranberries

Total N, gm.	Uric Acid, gm.	Solubility of Uric Acid		Diet
		Total gm.	Residual gm.	
10.24	.....	.....	....	Cranberry-sauce, 100 gm., each meal.
9.34	.....	.....	....	
9.24	.....	.....	....	
9.82	.....	.....	....	
9.55	.....	.....	....	
8.86	.....	.....	....	Cranberry-sauce, 200 gm., each meal.
8.24	.....	.....	....	
10.45	.....	.....	....	Cranberry-sauce, 100 gm., each meal.
10.52	.....	.....	....	
14.03	.....	.....	....	
9.53	.....	.....	....	
10.21	.....	.....	....	
10.02	.....	.....	....	Cranberry-sauce, 200 gm., each meal.
9.89	.....	.....	....	
9.87	.....	.....	....	Cranberry-sauce, 100 gm., each meal.
11.42	.....	.....	....	
10.48	.....	.....	....	
10.36	.....	.....	....	
9.28	.....	.....	....	Cranberry-sauce, 200 gm., each meal.
7.95	.....	.....	....	
10.60	0.362	0.293	—0.069	Cranberry-sauce, 100 gm., each meal.
10.52	0.355	0.242	—0.113	
11.10	0.389	0.207	—0.182	
10.13	0.361	0.320	—0.041	
9.72	0.362	0.171	—0.191	
9.38	0.329	0.182	—0.147	Cranberry-sauce, 200 gm., each meal.
8.53	0.292	0.060	—0.232	

stancy of acid excretion, the H^+ will vary inversely with the phosphate. These investigators believe that in the least acid samples there must have been greater amounts of phosphate excreted. The explanation offered follows: If the amount of phosphate claiming excretion is large and the body does not need to get rid of abnormally great amounts of acid, then it must eliminate a urine low in H^+ in order not to bring about general alkalinity of the body. A glance over our tables shows that the titratable acidity and the H^+ maintain a general relationship. This is well illustrated by the change from the prune to the cantaloup diet (Tables 8 and 10). Subject B on the last prune-diet day gave a H^+ of 4.80 and a value for A of 578 c.c. On the last cantaloup-diet day these became 7.38 and 250 c.c., respectively. Many more examples of this similarity in behavior are disclosed by our data.

TABLE 24.—THE H^+ OF THE URINES OF THIRTY VEGETARIANS

6.90	6.90	6.30
6.00	5.70	7.43
7.43	6.30	7.38
6.30	7.38	6.70
6.30	6.30	5.70
7.48	6.70	5.70
5.30	7.14	5.30
6.90	6.30	7.38
7.43	5.70	6.70
7.43	7.48	7.38

Average, 6.64

THE RELATION OF H^+ TO URINARY AMMONIA

Our results show, almost without exception, that these two factors are intimately related. An increase in H^+ is accompanied with an augmented ammonia production and vice versa. This sequence is well illustrated by the change from the highly acid prune diet to the basic cantaloup diet (Tables 8 and 10). Subject I showed values of 4.80 for H^+ and of 0.635 gm. ammonia N on the last prune-diet day. On the last cantaloup-diet day these were 7.38 and 0.188 gm., respectively. The other subject responded in a like manner. Other cases were observed in which these relationships held; the well-known, exceptionally low values for ammonia N were found after ingestion of alkalies. Examples of an increased ammonia output, with an increase in H^+, were observed in the benzoic acid experiment and in the prune, plum, cranberry and rice studies. These marked changes in ammonia excretion show that this factor plays an important rôle in the preservation of body neutrality. We believe that ammonia plays a greater part in checking the acidity of body fluids than Henderson and Palmer[5] assign to it. They state that their investigations prove conclusively that in normal persons nearly if not quite all the

TABLE 25.—H^+ ION CONCENTRATION AND THE CARBON DIOXID TENSION OF THE ALVEOLAR AIR

Date	Diet	Subject B		Subject I	
		H^+ Urine	CO_2 Tension mm.	H^+ Urine	CO_2 Tension mm.
July					
14	Basal diet	5.30	35.4	5.50	43.6
15	Prunes	5.30	34.0	5.30	39.5
16	Prunes	5.30	33.2	5.30	40.0
17	Prunes	5.30	33.1	5.30	38.9
19	Prunes	4.80	34.1	4.80	39.1
20	Cantaloup	5.30	37.0	5.70	42.8
21	Cantaloup	6.30	36.7	6.50	42.8
22	Cantaloup	7.38	38.2	6.90	43.8
23	Cantaloup	7.14	38.2	7.38	42.0
24	Cantaloup	7.38	37.6	7.38	41.8
26	Dasheen flour...	5.70	35.9	6.00	43.5
27	Dasheen flour...	5.30	35.0	4.90	43.6
28	Dasheen flour...	5.70	35.2	4.90	41.0
29	Dasheen flour...	5.70	35.1	5.30	41.0
30	Dasheen flour...	5.50	35.9	5.50	40.3
31	Dasheen flour...	5.30	32.0	5.30	40.0
Aug.					
2	Vegetarian	5.70	33.6	5.70	41.3
3	Vegetarian	5.30	33.0	6.90	42.3
4	Vegetarian	5.30	31.3	6.00	41.7
5	Vegetarian	5.50	33.2	5.70	41.3
6	Vegetarian	5.30	33.4	5.50	41.2
7	Vegetarian	5.70	33.2	5.70	40.3
9	Tomatoes	5.70	34.0	5.70	40.7
10	Tomatoes	5.70	34.5	5.70	39.9
11	Tomatoes	6.00	32.6	5.70	40.9
12	Tomatoes	5.70	35.0	5.85	39.9
13	Tomatoes	6.00	31.5	5.70	41.0
14	Tomatoes	6.00	32.6	5.70	39.0
16	Celery	5.70	32.6	5.30	40.1
17	Celery	5.30	33.9	5.30	39.4
18	Celery	5.50	31.4	5.50	42.0
19	Celery	5.30	32.0	5.30	41.0
20	Celery	5.30	33.1	5.30	39.7
21	Celery	5.30	32.5	5.30	39.1
23	Plums	5.70	33.0	5.50	42.2
24	Plums	5.30	32.5	5.30	42.1
Sept.					
1	Dates	5.30	34.7	5.30	41.5
2	Dates	5.50	33.3		
9	"Nuttolene"	5.70	35.6	5.70	40.5
10	"Nuttolene"	5.70	33.7	5.30	41.2
11	"Nuttolene"	5.70	32.7	5.50	40.5
13	Cauliflower	6.00	33.1	6.00	42.5
14	Cauliflower	5.50	33.1	6.00	42.7
15	Cauliflower	5.50	32.7	5.50	43.6
16	$NaHCO_3$, 10 g..	6.70	36.7	7.38	43.1
17	$NaHCO_3$, 15 g..	8.00	36.7	8.00	43.5

final regulation of H^+ through excretion falls on the phosphates and that the ammonia does not materially vary for purposes of regulating the reaction of the blood. The importance of the phosphates and bicarbonates in maintaining body neutrality is not minimized by us, but we feel that the significant changes in ammonia excretion observed indicate that this factor exerts a decided influence in this direction.

THE RELATION OF H^+ TO VOLUME

In their most recent publication, Henderson and Palmer[5] have reported an apparent relationship between urine volume and extent of acidity. Their averages showed that, with an increase in volume, there resulted a decrease in titratable and true acidity. While this does hold for their averages, yet a close inspection reveals many individual exceptions among their different cases. Our results do not permit of this conclusion. A good example of the non-relationship of these two is found in the case of Subject B (Table 10). His volume on the second prune-diet day was 1,700 c.c., and on the following day was only 710 c.c., while the H^+ remained the same on both dates. The last day of this period showed a volume of 1,520 c.c. and a higher H^+ of 4.80. Numerous other illustrations of this lack of relationship are to be observed. Our data can hardly allow of the conclusion that has been offered by these investigators. Haskins[26] has also failed to find any relation between the concentration of urine and its acidity. In all experiments on urinary acidity, the food factor must be carefully considered. If this is not done, erroneous conclusions are liable to be drawn.

THE RELATION OF H^+ TO THE SOLUTION OF URIC ACID

Early in this study, a close relationship between H^+ and the extent of solution of uric acid became apparent. The regularity with which urines having a greater H^+ than 7.00 deposit uric acid, while the alkaline urines dissolve this substance, is striking. Very acid urines are greatly supersaturated, as is shown in Tables 7 and 8. On the other hand, the heightened capacity for dissolving uric acid is to be seen in the alkaline urines of Tables 2 and 3. Specimens which are practically neutral in reaction are usually saturated. Such samples will sometimes experience precipitation, while in others solution will take place; but the amount is never large in either direction. The extent of the solution of uric acid in urine appears to be chiefly a function of the H^+. We have been able to confirm the observations of Hindhede and in a measure to explain the nature of this phenomenon. As has been mentioned, this worker found a relationship existing between titratable acidity and the capacity for the solution of uric acid. He suggested that since urines derived from diets containing certain vegetables, particularly the potato, were able to bring this substance into solution, the cause of the phenomenon might be found in the character of the ash of these foods. No experimental evidence for this was given. Our data show that such is really the case. Foods with an excess of base-forming elements lead to the production of a less acid urine. If the reduction is enough to result in an alkaline urine, then a heightened capacity for the solution of uric acid is obtained. Hindhede intimated that the volume of the urine might play a part in this phenomenon. Our results indicate that ordinary variations in urine volume exert an insignificant influence. It may well be, however, that this factor enters materially, when such large excretions (from 3 to 4 liters) as were obtained by Hindhede are dealt with.

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[26] Haskins: Proc. Am. Chem. Soc., Science, New Series, 1913, xxxviii, 679.

That urine generally contains more than enough uric acid for saturation is well known. There have been many attempts to determine the factors responsible for this condition. The advent of colloidal chemistry has stimulated some investigators in their efforts to apply it in explanation of the supersaturation of uric acid solutions. Among others, Schade and Boden[27] have been enthusiastic supporters of the colloidal theory. They believe that supersaturated solutions of this substance are made possible by the formation of a uric acid colloid. Recently, however, Kohler[28] has reported experiments which indicate that supersaturated solutions of sodium urate and of uric acid are true solutions and not of a colloidal nature. He arrived at these conclusions by dialyzing a supersaturated solution of sodium urate against a saturated solution. At the end of twenty-four hours, the two solutions were of equal concentration. Furthermore, the electrical conductivity of supersaturated solutions of sodium urate was practically equal to that of unsaturated or saturated solutions. Had the supersaturated solution been of a colloidal nature, its conductivity would have been greatly diminished. This investigator admits the possibility of the colloids being a factor but assigns to them a small part in the phenomenon.

PRACTICAL CONSIDERATIONS

The data presented make it clear that certain vegetables and fruits, on account of their preponderance in basic elements, lead to the formation of less acid urines. In this, their action is comparable to that of sodium bicarbonate and citrate, which are widely used in therapeutics. The question arises, can these efficient fruits and vegetables render similar service in therapy? If the desired ends can be obtained by a proper choice of diet, then a valuable step has been taken. Some of the possible applications of diet are, herewith, presented.

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[27] Schade and Boden: Ztschr. f. physiol. Chem., 1913, lxxxiii, 346; ibid., 1913, lxxxvi, 238.

[28] Kohler: Ztschr. f. klin. Med., 1913, lxxviii, 205.

NEPHRITIS

One of the current but much controverted theories of nephritis is that of Fischer.[29] This author believes that all the signs and symptoms of this disease are referable to changes in the condition of the kidney colloids. The cause of the pathological condition, according to Fischer, is an abnormal production or accumulation of acid in the kidney. The presence of acid is said to favor the solution of the kidney colloids; hence the albuminuria. Its action in making the kidney colloids swell is assumed to explain the increase in size of this organ, and further, it precipitates a second colloid making the change in color (graying) of the kidney. That there is an increased amount of urinary acid in nephritis is shown by the work of Henderson and Palmer.[2] These experimenters obtained an average value of 5.33 for the H^+ of the urine of cardiorenal cases, in contrast with the normal figure of 5.98. The work of Lewis, Ryffel, Wolf, Cotton and Barcroft[30] shows that in cardiorenal cases there is often a marked acidosis, with an accompanying fall in the CO_2 tension of the alveolar air.

If this acid theory of nephritis is true, then the treatment indicated is the introduction of bases in order to overcome this increased acidity. Fischer[31] has reported much success in the treatment of nephritics by alkalies. That patients with kidney lesions differ in their response to the administration of alkalies has been shown by Palmer.[32] He reports that it is difficult to reduce urinary acidity in many nephritics by alkaline treatment, but that after the urine has been rendered alkaline and then allowed to become acid, the patients respond normally to further administration of alkalies. The danger is also pointed out by this author, of pushing the reaction beyond that of the blood, for then albuminuria is liable to follow.

Fischer has always advocated the use of vegetables and fruits and, it now appears, with good reasons. Many of these foods are markedly efficient in producing less acid urine. That a food is a fruit or a vegetable, however, is no indication that the eating of it will decrease the acid formation. Thus, we have been able to show that prunes, plums and cranberries cause an increased acid production rather than the expected decrease. This we have attributed to their content of benzoic acid. On the other hand, dietaries made up largely of potatoes with smaller amounts of apples, bananas, raisins and oranges offer an excellent means of introducing the desired bases into the body. These foods may all be obtained throughout the year and thus have a special advantage. A very suitable base-yielding food, which can only be had during the summer months, is the cantaloup. The tomato is also valuable. Care should always be taken not to eat too much of the cereals and meats, which have a predominance of acid-forming elements.

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[29] Fischer: Nephritis, New York, 1912.

[30] Lewis, Ryffel, Wolf, Cotton and Barcroft: Heart, 1913, v, 45. Lewis, Ryffel, Wolf, Cotton, Evans and Barcroft: Proc. Physiol. Soc., Jour. Physiol., 1913, xlvi, 53.

[31] Fischer, Martin H.: The Treatment of Nephritis and Allied Conditions, Jour. Am. Med. Assn., 1913, lx, 1682.

[32] Palmer: Boston Med. and Surg. Jour., 1913, clxix, 464.

URIC ACID CALCULI

Uric acid concrements are estimated to comprise from 60 to 81 per cent. of all the varieties of urinary calculi. The formation of a given kind of calculus is, obviously, dependent on the reaction of the urine. A high urinary acidity favors the formation of uric acid calculi. Thus, Henderson[6] has shown that at the more acid reactions uric acid is present mostly in the free condition. As the H^+ decreases, more and more of the substance is in the form of urates. Our results show that acid urines are always supersaturated with uric acid and that they will experience precipitation when opportunity for the establishment of an equilibrium is given. On the other hand, alkaline urines are always capable of dissolving added uric acid, showing that as excreted they are not saturated. Kohler[28] has demonstrated that supersaturated solutions of uric acid experience spontaneous precipitation much more readily than do those of sodium urate. The correct prophylactic measures are therefore evident. In order to prevent a tendency to the formation of uric acid calculi, it is necessary that the urine be of low acidity and that the purin intake should not be too great. The first of these conditions can be fulfilled by the ingestion of alkalies and just as efficiently by eating some of the many suitable fruits and vegetables. These have a further advantage in that their purin content is negligible and therefore reduces to a minimum the exogenous output of uric acid. The value of such diets in the treatment of persons subject to uric acid calculi is also apparent.

SUMMARY

The composition of the urine is markedly influenced by the character of the food intake. Foods which have a preponderance of basic elements lead to the formation of a less acid urine. Conversely, foods with a predominance of acid-forming elements increase the urinary acidity. Potatoes, potato ash, oranges, raisins, apples, bananas and cantaloups are very efficient in reducing the acid output. Tomatoes are of less value in this respect. Rice and whole-wheat bread increase urinary acidity. Plums, prunes and cranberries, although yielding a basic ash, nevertheless increase the acid formation. The latter foods contain benzoic acid, which increases the acidity owing to the formation of hippuric acid.

An increase in H^+ is accompanied by a significant increase in the ammonia excretion. Our results indicate that ammonia exerts an unmistakable influence in the preservation of body neutrality.

Titratable acidity and H^+ are closely related in behavior.

A lack of relationship between H^+ and urine volume is pointed out.

The solubility of uric acid in urine is closely dependent on the H^+. All urines with an acid reaction are supersaturated with uric acid, while alkaline urines are capable of dissolving added uric acid.

Further evidence for the interrelationship of the alveolar CO_2 tension and the H^+ of the urine is submitted and their dependence on diet is pointed out.

The H^+ of the urines of thirty vegetarians was 6.64, in contrast with the value of 5.98, reported by Henderson and Palmer, for persons living on a mixed diet.

I take pleasure in expressing my thanks to Prof. Lafayette B. Mendel for his kindly encouragement and advice during the progress of this and other work. I am also indebted to Prof. Harry W. Foote for the use of his solubility apparatus.

BOOK REVIEWS

A Practical Treatise on Medical Diagnosis for Students and Physicians. By John H. Musser, M.D., LL.D. Sixth edition, revised. Illustrated with 196 engravings and 27 colored plates. Lea and Febiger, Philadelphia and New York, 1913.

The sixth edition of Musser's "Diagnosis" is worthy of more than the usual perfunctory notice and congratulations. The gradual enlargement of the previous editions threatened the usefulness of the work. Condensation in such a case is obviously difficult, but this time it has been carried out with such excellent results that a long period of popularity seems assured. Although the text has been largely rewritten, it still retains the features that made it peculiarly valuable as a text-book and work of reference for physicians and students of medicine. It is full of the ripe knowledge of a keen observer and thoughtful and critical student, who combined with an unusual experience among all kinds of illness an enthusiastic love of collating, comparing and analyzing his own observations and those of others. In all the sections in which examination of patients is important, a wealth of information can be found. This should make the work especially valuable to beginners in clinical work, who may well turn to it as a commentary and guide to the puzzles and problems of the wards and out-patient department. The laboratory sections have been brought well up-to-date; their directions are clearly stated, and they also will be useful, though in many cases the student will need a more special handbook, as well as practice under a keen teacher in the preclinical work. Some comments on individual features are warranted. The section on "Diatheses" might with advantage be replaced with a new one in which the modern view of constitutions could be briefly set forth. Roentgenography is evidently getting to be too extensive to be included in a text-book on diagnosis, or if it is, must be handled by one who includes x-ray work in his daily activity. The relative value of the Widal test, that is, its lack of value as an early diagnostic factor, might have been more explicitly stated; there is still far too much reliance on it. The statement in regard to movable dulness in serofibrinous pleurisy seems to have come down from earlier days, and the description of the Grocco sign is correspondingly vague. Many of the additions to laboratory methods are inadequately indexed, but can be found if looked for. There seems need of a better test than the one given for chlorids in urine, however, now that that is of therapeutic value; also of more attention to nitrogen determinations, ammonia, urobilogen, diastase and oxybutyric acid. Blood viscosity would seem worthy of consideration. The statement in regard to embryocardia, page 274, that it is "heard in all conditions which cause a pronounced rapidity of the heart (over 120)," etc., is at least imperfectly expressed. The numerous illustrations are for the most part well chosen. That of Heberden's nodes represents a very remarkable condition and one we should like to know more about, but it does not give a correct idea of what Heberden and most writers after him had in mind. The diagram opposite page 704 gives a wrong impression of the enlarged veins in ascites with portal obstruction. The legends of the two taenia pictures, page 432, are transposed. These are but trifling defects in a work so full of accurate information as the new "Musser," and it may be confidently expected that within a short time the industrious author will more than make up for inevitable defects in a book dealing with so many and so diverse facts. Mechanically the work is well done.

Blood-Pressure in Medicine and Surgery. A Guide for Students and Practitioners. Edward H. Goodman, M.D.

At the present time there is no good book on blood-pressure in the English language. Yet the subject is one of vital and pressing interest. One looks with eagerness, therefore, on each new book, hoping that here at last one will find the essential combination of scholarship, clinical experience, and good judgment.

Unfortunately the latest work by Goodman is once more a disappointment. It is a conscientious, though far from complete, compilation of the literature on blood-pressure, but the material is not critically examined. Good, bad and indifferent, old and new articles receive almost equal consideration and (apparently) approval.

The author's own observations on the clinical side of the subject are good so far as they go, but are too few to add much to his summary of others' work.

On page 72 Féré is quoted as saying that attacks of cholera are dangerous to individuals suffering with disease of the arteries. Presumably "cholera" means choler, or anger. Similar slips are scattered through the book. Despite these limitations the book would be of use to any one who wishes to read a summary of a good deal that has been written on the subject of blood-pressure within the past fifteen years.

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THE RELATION OF METHODS OF DISPOSAL OF SEWAGE TO THE SPREAD OF PELLAGRA *

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INTRODUCTION

In analyzing the data collected in 1912 we were struck by the inequalities in the distribution of pellagra in the townships of the county in which our studies were undertaken. This rate ranged from no cases to 71 cases per ten thousand of population. A further analysis, based on the distribution of the population in rural, urban and mill-village communities showed that in rural communities the incidence rate was 16 per ten thousand of population; in the city of Spartanburg, exclusive of the mill-village section, it was 29 per ten thousand, while in the mill-village communities it averaged 104 per ten thousand.

One of the features of our epidemiological work in 1912 was the investigation of methods of disposal of human wastes. In certain sections of the city of Spartanburg a water-carriage system was in use. In the rural districts the white farmers usually had the unscreened surface privy. Privies were but seldom found on the premises of negro tenants on the farms. In the cotton-mill villages, about twenty-eight in number, in which pellagra prevailed much more extensively than elsewhere, two types of privy were in use. The usual type was an unscreened wooden outhouse where the excreta were deposited on the ground. In a few mill-villages the privy type was an unscreened wooden outhouse provided with metal containers for the reception of fecal matter. In some of the villages scavengers cleaned the privies at weekly intervals; in others at less frequent inter-

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* This paper forms a part of the Second Progress Report of the Thompson-McFadden Pellagra Commission of the New York Post-Graduate Medical School and Hospital. It was presented in part before Section K of the American Association of Science, at Atlanta, Ga., Jan. 2, 1914.

vals or irregularly. These villages were laid out in streets, the dwelling-houses were but short distances apart and the privies necessarily in close proximity to the dwelling-houses. No effort was made to screen these privies; flies and other insects had free access to the collections of excreta.

In a series of 243 cases of pellagra investigated in 1912, the following methods of disposal were found to be in use.

In 5 per cent. (11 of the cases) a modern water-carriage system was used.

In 20 per cent. (51 of the cases) the unscreened wooden outhouses were equipped with metal receptacles.

In 60 per cent. (152 of the cases) the privy was an unscreened wooden outhouse, the feces being deposited on the ground.

In 12 per cent. (29 of the cases) there were no privies.

The average sanitary index of the privies used by these 243 pellagrins was 16 per cent., computed in accordance with the methods adopted by the Rockefeller Sanitary Commission. The existing sanitary conditions were summarized as follows:

> The most insanitary condition found in the county is the absence of properly constructed privies. Outside of a part of the city of Spartanburg, which is supplied by a water-carriage sewage system, there is no effective provision in the county for the proper disposal of human excreta. A second striking insanitary condition is the almost complete absence of effective screening of dwellings. These two conditions present a situation highly favorable to the transmission of disease organisms eliminated in the excreta, both by direct contamination of food and persons and by insects. This situation is naturally aggravated in the mill villages and small towns by the greater congestion of houses.

As a result of these, as well as other observations, the members of the commission were impressed with the possibility that insanitary methods of disposal of human wastes might be an important factor in the spread of pellagra.

In the course of our investigations during 1913 it was determined that this feature should receive special attention. The principal point which we endeavored to ascertain was whether the endemic foci of the disease were more common in those communities and in those sections of cities in which the methods of disposal of human wastes were insanitary. In order to ascertain these facts we made a careful study of the exact distribution of the cases of pellagra, including a history of its distribution in the past. By personal visits to pellagrins and their relatives it has been possible for us to secure definite information as to the exact residence of pellagrins. We have endeavored to locate precisely all houses occupied by them from the inception of the disease up to the present time. The information relative to residence, as well as to other pertinent facts, has been transferred to our pellagra "house card," a copy of which we show. These "house cards" have been filed by locality and from them we have constructed spot-maps showing the exact location of the cases of pellagra of which we have record.

HOUSE CARD

House No. *190.* Township, *Spartanburg. Whitney.*
Loation, *Whitney Mills No. 101.*
Pellagrous History Date, *July, 1913.*

No.		First Erythema (Place, Date)
86.	Here from *Jan. 22, 1912,* to *early in 1913.*	*July, 1911. Clifton No. 2. H. No. 191.*
87.	Here from *Jan. 22, 1912,* to *early in 1913.*	*June, 1912. Here.*
88.	Here from *Jan. 22, 1912,* to *early in 1913.*	*June, 1912. Here.*
89.	Here from *Jan. 22, 1912,* to *early in 1913.*	*June, 1912. Here.*

Notes—*86 = mother; 87 = son; 88 = daughter; 89 = daughter.*
Apparent house of origin for Cases *Nos. 87, 88, 89.*
House: Construction—*Frame.* Screened, *no.*
Sanitary Condition—Good, poor, *fair.*
Water Supply—Well: *dug, with bucket. Deep.* Surface contamination, *no.*
Disposal of Excreta—Privy type: A, B, C, D, *E,* F. Distance from house, *twenty yards.*
Method of Ultimate Disposal—*Scavenger.*
General Sanitary Condition—*Poor.*

In all the mill-villages in Spartanburg County unscreened surface privies were in use and, in order that possible contrasts in the prevalence of pellagra might be shown, for the purpose of this study, it was necessary to extend our investigations to industrial communities elsewhere in the state in which water-carriage systems of sewage disposal had been installed. Through the state health authorities we learned that four cotton-mill villages in the state had adopted such methods of disposal. In two of these villages, one situated in Greenville, S. C., and the other in Union, S. C., the water-carriage sewer systems had been installed less than a year. The third was the Newry mill-village in Oconee County in the western part of the state, in which a water-carriage system of sewage disposal was installed about twenty years ago. The fourth village was the Republic mill-village in Chester County, situated in the northern part of the state, in which a water-carriage system was installed about three years ago. We have investigated the sanitary condition and prevalence of pellagra in the Newry mill-village and the Republic mill-village. As the water-carriage system of disposal had been in use in the other two villages for only a few months, their investigation was left to the future.

This study has enabled us, first, to compare the prevalence of pellagra in sewered and unsewered portions of the city of Spartanburg, and second, to make a similar comparison of two entirely sewered villages with a large number of villages where unscreened surface privies were in use.

THE SPREAD OF PELLAGRA IN THE CITY OF SPARTANBURG AND ITS RELATION TO VARIOUS METHODS OF DISPOSAL OF HUMAN WASTES

We have record of a total of 268 cases of pellagra in the city of Spartanburg (204 white and 64 colored). This includes not only the pellagrins living there in 1913 but also all known cases which have occurred. Of these 268 cases, 168 are known to have originated in the city.

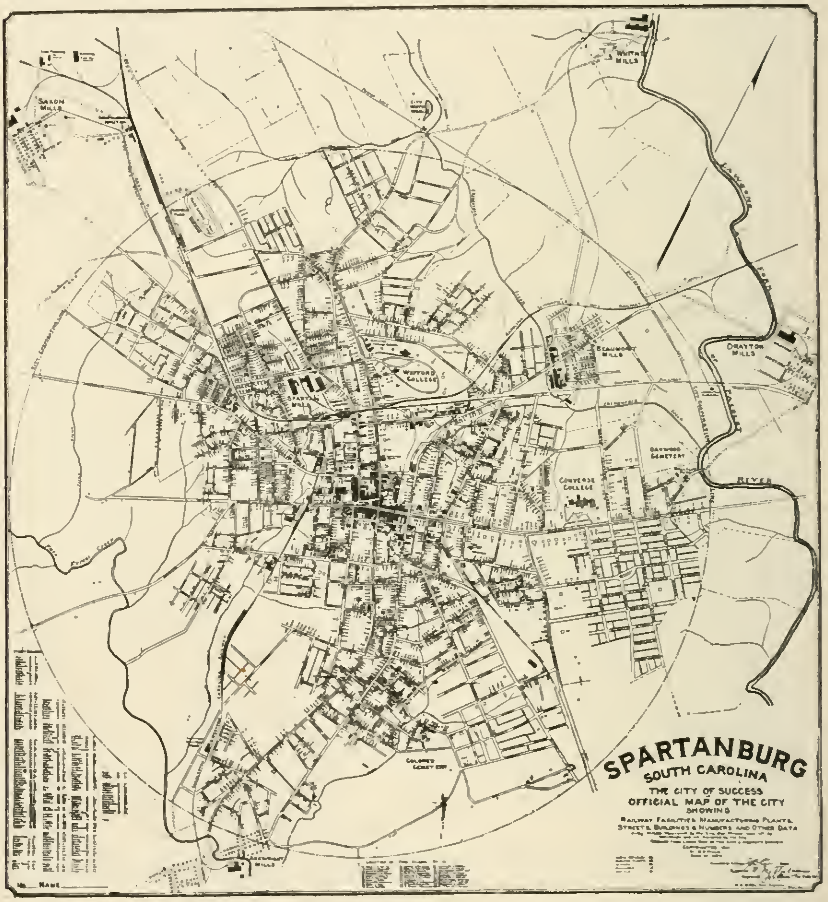

Fig. 1.—Map of Spartanburg, S. C., showing plan of city.

Figure 1 is a reproduction of the plan of the city, the black portion in the center indicating the location of the business section. It will be noted that there are three mill-villages within and two just beyond the city limits. The largest cotton-mill is situated in the northwestern quadrant, the next largest in the northeastern, and the smallest in the southwestern quadrant.

Figure 2 shows the geographical distribution of pellagra in the city of Spartanburg together with the distribution of the sewer mains. It is clearly evident from this map that practically all the active foci of the disease were in the sections of the city in which unscreened surface privies were used. Two active foci of the disease existed in the southeastern quadrant of the city. One of these foci was in a sewered district but none of the pellagrous houses had sewer connections. The other was in a negro settlement and all the privies in this vicinity were of the unscreened surface type.

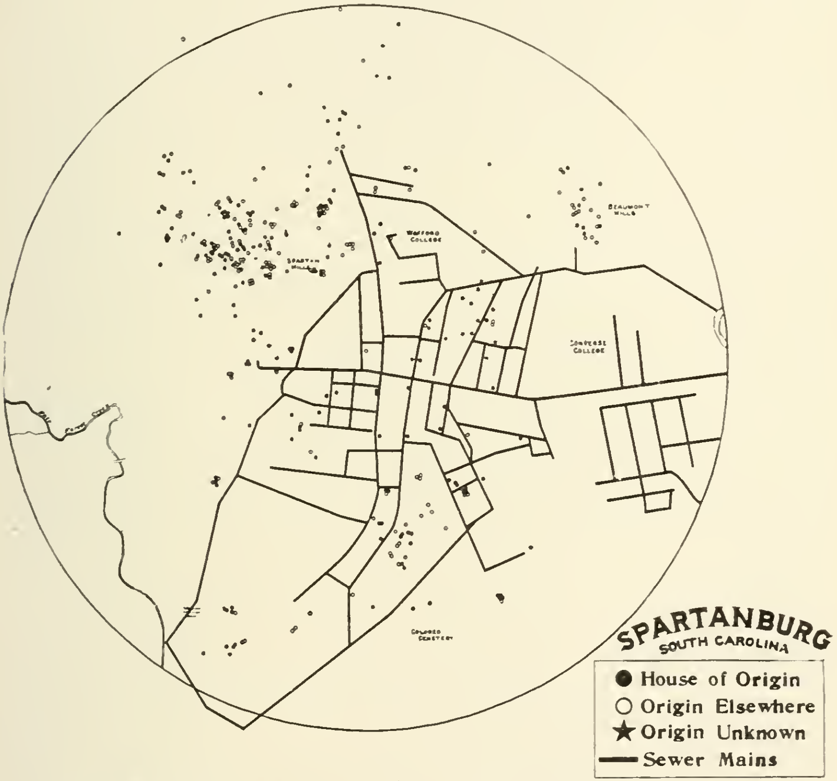

Fig. 2.—Map of Spartanburg, S. C., showing distribution of cases of pellagra in the city and distribution of sewer mains. Note that 168 of these cases originated in the city and that the largest number of cases occurred in the northwestern quadrant.

Of the total 268 cases of which we have record in the city of Spartanburg, we have investigated methods of disposal of excreta in 241 instances. In 230 cases, or 95 per cent. of the total investigated, the method of sewage disposal was the unscreened surface privy. In eleven instances, or 5 per cent. of the total, a water-carriage system of disposal was in use. A detailed examination of the records of these eleven cases has shown the following facts:

Patient 12 was a white woman, aged 40, married, whose residence had not changed since 1907. She contracted pellagra in 1911 and had mild recurrences in 1912 and 1913. She lived on one of the better residential streets; her circumstances were comfortable, and her only occupation was housework. The house was not screened. She gave no history of association with pellagrins, except visits to two persons suffering with pellagra. Our records, however, show that in a house immediately in rear of hers, Patient 496, a colored female, died of pellagra, July 12, 1910, and that during 1911, Patient 379, a colored female, lived directly across the street from her. The houses of these two colored pellagrins had the usual unscreened surface privies.

Patient 19, a white man, aged 20, contracted pellagra in the spring of 1911. His residence remained the same from 1905 to the fall of 1912. His home was in the best residential section of the city and the house was effectively screened. He was a college student and his circumstances were comfortable. His general health was good until the summer of 1909, when he contracted malaria in a saw-mill camp in South Carolina, near Augusta, Ga. Since that time his health has not been good. In 1910 he again spent five months (January to June) in a lumber-camp near the Georgia state line in South Carolina. This patient had severe recurrences during 1912 and 1913. No cases of pellagra have been located in the vicinity of his home in Spartanburg and there is no history of association with pellagrins.

Patient 24, a white man, aged 61, contracted pellagra in May, 1912. His residence had remained the same for several years. His home was situated in a good residential section but was not screened. He had been engaged in clerical work since 1881; his health had been good, and his circumstances appeared to be comfortable. He gave no history of association with pellagrins and we were not able to locate any cases in the vicinity of his residence. The disease recurred in 1913 and he died in the fall of 1913.

Patient 40, a white woman, aged 63, contracted pellagra in February, 1912. She had not changed residence for two years except for prolonged visits to relatives in South Carolina. Her home was situated in the business section of the city and, though it was screened, the screening was not effective. Two visits were made to this home by members of the commission. We found the screens raised, swarms of flies present and the general sanitary conditions very unsatisfactory. The patient's economic circumstances were poor and her occupation was housework. Patient 342, a white woman, who was a chronic pellagrin, lived with her during August, September and October, 1911. This patient (No. 40) died of pellagra, Aug. 10, 1912, having first shown symptoms in February, 1912.

Patient 57, a white woman, aged 43, first showed symptoms of pellagra on June 27, 1912. She lived in the best residential section of the city and had been living there since 1910. Her home was effectively screened and her husband well-to-do. She apparently had had some gastric disorder for a number of years. Her only occupation was the supervision of her housework. There was no history of association with pellagrins and no pellagrins lived in the immediate vicinity. There was no definite recurrence during 1913.

Patient 59, a white woman, aged 38, contracted pellagra in March, 1909, three months after confinement. Her home was in one of the best residential sections of the city and she had been living there since 1907. Her house was screened in part and her circumstances were those of comfort. There was no history of pellagra in her family and she gave no history of association with pellagrins. She had slight recurrences in 1910, 1911, 1912 and 1913.

Patient 208, a white man, aged 59, developed pellagra in December, 1909, and severe symptoms persisted until September, 1910. He had completely recovered from the attack in November, 1910, since which time his health had been good. The house of origin for this case was situated in one of the better residential sections and he lived there from 1907 to early in 1912. His circumstances were those of comfort and his health prior to the development of pellagra had been good. No pellagrins have been located as living in the immediate vicinity of the house of origin for this case. For several years it had been the patient's custom to make visits from time to time to relatives in certain country districts in the county. One of his sisters died of pellagra in 1907 and another died of pellagra in 1910. Both were living in Spartanburg county and were well-to-do. His health has been excellent since November, 1910.

Patient 233, a white woman, married, contracted pellagra in May, 1910, in a house located in the best residential section of the city. She lived in this house from August, 1908, to March, 1911, and it was not screened. Her husband was well-to-do and she had enjoyed good health prior to the development of pellagra. Symptoms recurred in 1911, but there was no recurrence in 1912 and 1913. We have no record of pellagrins living in her immediate vicinity during 1909 and 1910. Her father-in-law (Case 321) died of pellagra in September, 1910, at his home in an unsewered portion of the city of Spartanburg and she had visited him there frequently during his illness.

Patient 426, a white woman, single, died of pellagra in 1902. Her father was well-to-do. Prior to the development of pellagra her health is said to have been good. She had been living in the same house for many years. As the condition was not recognized as pellagra until several years after her death the history is very incomplete. Evidence of association with other cases of pellagra is wholly lacking.

Patient 541, a white woman, aged 21, married, first showed erythema in April, 1913, though she gave history of ill health since November, 1912. She had lived since 1909 in a good residential section and the house was screened in part. Her circumstances were those of comfort and her health had been good prior to November, 1912. The nearest case of pellagra was a white man, Patient 5, who contracted pellagra in 1911 and died of it in 1912 in a house directly across the street. His house was provided with an unscreened surface privy. This woman, Patient 541, made a visit of three weeks' duration, during the summer of 1912, to relatives in a southern state on the gulf coast where surface privies were in use. There was no history of association with pellagrins.

Patient 755, a white man, aged 32, druggist, contracted pellagra in the spring of 1913 and died of it in August, 1913. During 1913 he lived in the best residential section of the city and was well-to-do. The history of this case is very incomplete and we have no data concerning association with pellagrins.

Of the eleven patients, three (Cases 12, 233 and 541) gave a history of living or visiting in the same house or next door to pellagrins using surface privies; another (Case 40) lived under very insanitary conditions in the same house with a pellagrin; and a sixth (Case 208) had two sisters who were pellagrins, one dying before he developed the disease and the other dying the following year. Two, Patients 24 and 755, were men engaged in occupations bringing them constantly into contact with the general public. In the other four cases, (Nos. 19, 57, 59 and 426) we have been unable to learn of any relationship whatever to antecedent pellagra.

THE SPREAD OF PELLAGRA IN UNSEWERED MILL-VILLAGES IN SPARTANBURG COUNTY

Our records show that pellagra occurred and has originated in all the mill-villages in Spartanburg County. In all these villages unscreened privies were in use. In some, the fecal material was collected in pails; in the majority, however, it was deposited on the ground. Many of these mills were situated but a few miles distant from one another and for a number of reasons there was a tendency for certain elements of their population to move, with great frequency, from one mill to another. This element of the population—generally termed "floating"—varied from about 15 per cent. to even more than 33 per cent. Cases of pellagra commonly occurred in this floating element and frequently it was necessary for us to trace pellagrins from mill-village to mill-village before finally locating them.

Six of these villages were selected for intensive studies and by house-to-house visitations all existing cases of pellagra were accurately located. The incidence rate for 1912 and 1913 was higher in some villages than in others, but incident cases occurred in every village during 1912 and 1913. It was observed that in several of these villages incident cases of pellagra would be quite numerous one season and relatively few the next, or *vice versa*.

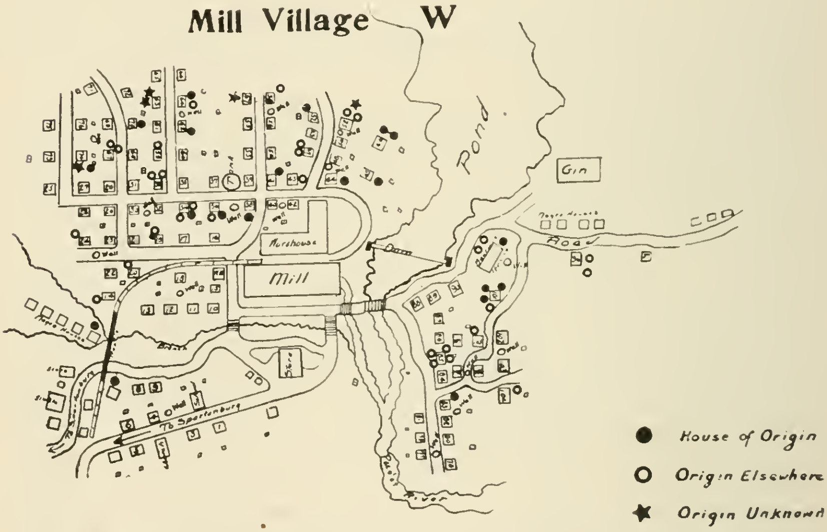

Fig. 3.—Distribution of pellagra in mill-village "W." Spartanburg County. Unscreened surface privies are shown as small blocks in the rear of dwelling-houses. Note that twenty cases of pellagra have originated in this village.

Spot-maps have been constructed of these mill-villages showing the distribution of pellagra. Figure 3 is a reproduction of the plan of village *"W"* showing the distribution of the disease, and this is a fair example of conditions in all these villages. It will be noted from this map that at least twenty cases of pellagra have originated in this village.

HISTORY AND SPREAD OF PELLAGRA IN MILL-VILLAGES IN WHICH WATER-CARRIAGE SYSTEMS OF DISPOSAL OF SEWAGE HAVE BEEN INSTALLED

Newry Mill-Village

This village is located in Oconee County in the northwestern part of South Carolina. It was built about twenty years ago, its first president being Captain Courtenay of Charleston. Captain Courtenay installed a water-carriage system of sewage disposal before the mill-village dwellings were occupied and this system has been continuously in use to the present time. The closets have been grouped (from four to eight in each group) in detached houses convenient to a group of dwelling-houses, rather than as individual closets in each dwelling-house. One of these privies has been assigned to each dwelling and the tenant held responsible for its proper care. The annual cost for repairs and upkeep has amounted to but little. The total population was 750. The operatives employed in the mill were recruited from the same sections and from the same elements of the population, as were operatives in mills elsewhere in the state. They were accustomed to eat the same foods as were eaten by the inhabitants of other mill-villages throughout the state. The company store was selling about a bushel of shipped (western) corn-meal to each family per month. The only respect in which their dietary habits possibly differed from those of some of the mill-villages in the state, lay in the fact that fresh meat could be obtained throughout the year from a local market. In some of the mill-villages in Spartanburg County fresh meat was not on sale in the village during the hot summer months. In others, however, where pellagra prevailed extensively, fresh meat could be obtained throughout the year from the mill-village market.

The Newry village is almost surrounded by permanent streams, and the eggs and larvae of *Simulium* were observed in abundance. The only demonstrable difference between this mill-village and (with three exceptions) those elsewhere in the state lay in the fact that a water-carriage system of sewage disposal was used there, while the method employed elsewhere was the unscreened surface or pail privy. With these facts in mind it is of interest to study the history of pellagra in this village.

In August, 1911, a woman showing active pellagrous skin lesions came into the village from Georgia. She departed the same day on the advice of the local physician and has since died.

In the spring of 1908, a family which had been resident in the village for several years, purchased a home near the railway (at Courtenay Station) about one mile away. They lived there until December, 1911. A woman with pellagra resided next door during the period of their residence at Courtenay Station. In June, 1909, the mother (Case 2064) contracted pellagra. In December, 1911, they returned to the Newry mill-village and were still there in October, 1913. The family consisted of six adults and one child. No other case of pellagra was observed in the family and this one case had recurrences in 1910, 1911, 1912 and 1913.

Another woman (Case 2060), one of the four patients now resident in the village, gave a rather indefinite history of pellagrous symptoms as far back as 1910. The family had been extremely migratory and it was difficult to determine with certainty exactly where and when the disease developed. She first lived in the village for two months in the winter of 1910 and it is possible that she was at that time pellagrous. The first positive history of the disease began in the spring of 1912, when a frank erythematous rash appeared. At that time she was living on a farm some ten miles north of the village. She returned to Newry in November, 1912, and lived there until the last of February, 1913. She was then away until June, 1913, when she returned to the village and was still there in September, 1913.

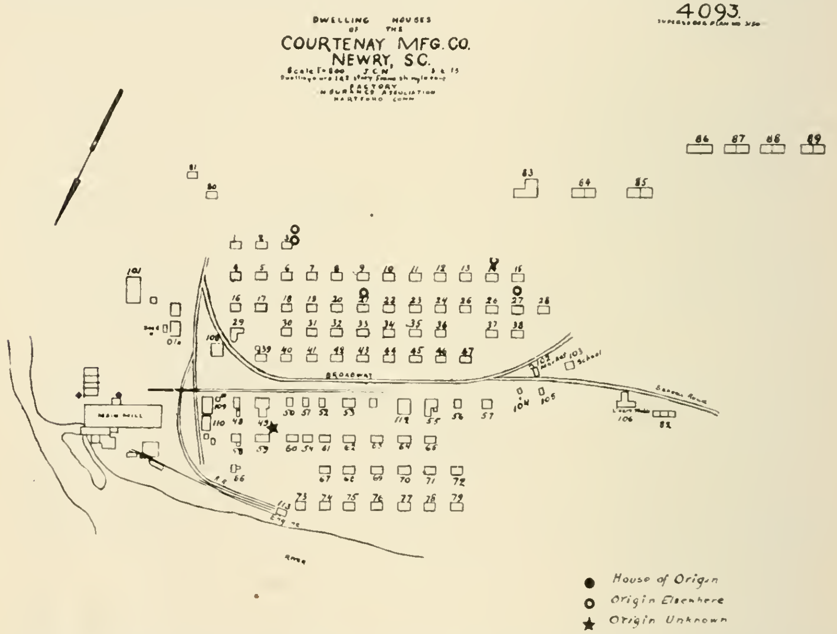

Fig. 4.—Distribution of cases of pellagra in the Newry mill-village having a water-carriage system for disposal of sewage. Note that all patients with one possible exception contracted pellagra before moving into the village.

Patient 2061, who had been a pellagrin since 1910 and had had a recurrence each succeeding year, first moved to the village in November, 1912, and still lived there in September, 1913. Her 10-year-old son (Case 2062) had pellagra, but he likewise developed the disease early in 1912 before coming to the village.

Case 2063, that of a young woman who died of pellagra in Newry, Aug. 17, 1913, offers the only instance of possible origin in this mill-village, inasmuch as she moved there late in the fall of 1912 (exact date not known) and apparently did not have any erythema prior to Aug. 3, 1913, two weeks before death. She was under treatment by the local physician for persistent diarrhea from June 6, 1913, and gave a history of stomatitis, diarrhea and general poor health the previous year, while living at another village in a house within a few doors of at least two other cases of pellagra. It seems highly probable that she was a pellagrin in 1912 before coming to Newry.

The history of pellagra in this village may be summarized by saying that five pellagrous patients have been observed, all of whom, with one possible exception, were suffering with pellagra on arrival in the village. No case of pellagra clearly originating in the village has been found. Figure 4 indicates the distribution of pellagra at the Newry mill-village.

As a control study we investigated the prevalence of pellagra in a mill-village on the outskirts of Seneca, S. C., about four miles distant from Newry mill. The methods of disposal of human wastes in this village were exactly similar to those observed in mill-villages in Spartanburg County (unscreened surface privies). We were shown active cases of pellagra originating in this village. The history of pellagra in this village as given us by the local practitioners indicated that it had prevailed here to much the same extent as in the mill-villages in Spartanburg County.

Oconee County had not so large a number of cotton-mills as Spartanburg County, nor were the mills in such close proximity one to another. For this reason the "floating" or transient population was appreciably smaller.

Republic Mill

This mill is situated at Great Falls, Chester County, in the north central part of South Carolina. It was built in 1910 and was put into operation in June of that year. There were ninety-two houses in the village and its population was between 475 and 500. Before building the village, Mr. R. S. Mebane, president of the mill, made a special study of the problem of the proper sanitation of mill-villages, especially with reference to the water-supply and sewerage. In building the village he constructed a complete sewer system, connected with every house, and placed in each house a kitchen sink and flush closet. The system differed from that at Newry Mill in that the closets were placed in the houses instead of being grouped outside. This mill-village was visited for two days in September, 1913, and as complete a study made of the pellagra situation as was possible in that time. Dr. J. B. McKeown, the resident physician, kindly gave practically the entire two days to assist in the inquiry. Notes were secured of six cases of pellagra concerned in the history of the village during the three years of its existence.

The first patient, a woman, aged 30, had lived in the village only four months. She was at about term with her fourth child. She had no erythema when seen and said she had never noticed any. When seen by us she had a very bad stomatitis and glossitis and had suffered with persistent diarrhea for the past nine months. She had lost considerable weight and had suffered recently with severe burning of feet. While, in the absence of any skin manifestation, it was not considered possible to make an absolutely positive diagnosis, we agreed with Dr. McKeown in considering hers a very probable case of pellagra. There was no question, however, but that her symptoms antedated her coming to Republic Mill.

A second case, not now in the village, was reported by Dr. McKeown as having moved there in January, 1913. The patient first came to his attention in April, 1913, when she was confined. About three weeks later he was again called and found her with all the symptoms of an active attack of pellagra — diarrhea, nervous symptoms, bad stomatitis, and a symmetrical erythema on the backs of her hands. She left the village the following June. In November we received a letter from Dr. L. S. Hay of Rock Hill, a former physician of the case, saying that she had been a pellagrin under his care the previous year before coming to Republic Mill.

The third patient was a woman who had been a pellagrin since the spring of 1909 and had lived in Republic Mill for the past two years. She had had no active symptoms of the disease during the period of her residence in the village.

The fourth case was that of a boy aged 8, who came to the village in March, 1913. When seen he showed no evidence of the disease except a symmetrical scaliness and pigmentation of the backs of the hands. In the spring of 1913 he had an erythema of hands and feet and an attack of sore mouth. He was seen by Dr. McKeown at that time and the case diagnosed as pellagra. According to his father's statement he had suffered an exactly similar attack, though rather more severe, in March of the previous year (1912) before coming to Republic.

The fifth case was that of a boy, aged 6, who, according to Dr. McKeown's statement, presented a typical erythematous rash of both hands and feet and was suffering from diarrhea and sore mouth and tongue during the summer of 1912. The erythema had appeared in June, 1912, and he had come to the village the preceding January. His mother stated that he had a somewhat similar rash the previous year (1911) before coming to Republic, which she thought was "poison oak." She said that she "broke out" the same way on her hands, face and neck about six years ago and that her mouth got sore. She also stated that her father was said to have died of pellagra. The woman was of a very low order of intelligence and her statements were not considered very reliable. A diagnosis in her case was not possible and the time of appearance of the boy's first symptoms could not be determined positively. The case must be considered as possibly originating at Republic Mill, but there was a fair probability that the boy was pellagrous prior to coming to the village.

The sixth patient, a colored woman, aged 35, did not live in the village proper, but in a cabin about one fourth of a mile distant. This was a typical case, but the patient had been living there only a short while and her symptoms clearly antedated her present residence.

Of the total six cases which could be discovered in the three years history of the village, only one could possibly have arisen there and in that case the evidence seemed to point to probable origin elsewhere. Figure 5 shows the distribution of pellagra in this village. Two of the cases cited above are not shown on this map because the patients lived beyond the limits of the village.

One significant fact in these two villages was the difficulty encountered in finding cases of pellagra even by careful search, whereas in all other villages studied, a few moments' inquiry was all that was necessary to locate many cases of pellagra, well known to the population at large.

The second striking feature, of course, was that, with two doubtful exceptions, each of the eleven patients affected with the disease who could be found in these two water-sewered villages had been pellagrins prior to coming to the respective villages. It appears, therefore, that while cases of pellagra had been introduced into these villages from time to time during their history of twenty and of three years, respectively, no case of the disease could be shown definitely to have originated in these villages and only two could possibly have done so.

With reference to Republic Mill, the point arises whether three years is not perhaps too short a time for pellagra to show itself in a newly constructed village. That this is not true is demonstrated in the case of a mill-village in Spartanburg County which was built in 1911 and went into operation in April, 1912. During the following year five known cases of pellagra moved into the village. Two children (Cases 546 and 585) who in July, 1912, moved into the house next door to one of these imported cases, developed typical pellagrous symptoms in May and June, respectively, the following year (1913). There were very clear histories of the absence of any previous manifestations of the disease. A third patient (No. 745), a boy of 7 years, developed pellagra in March, 1913. There was no history of a previous attack. The immediate connection with a previous case of pellagra appeared to be the boy's grandfather, who was a pellagrin and had visited the family during the previous winter. The development of these three cases within little over a year after the newly built village was first occupied would argue strongly that the apparent freedom of Republic mill-village from pellagra cannot be explained

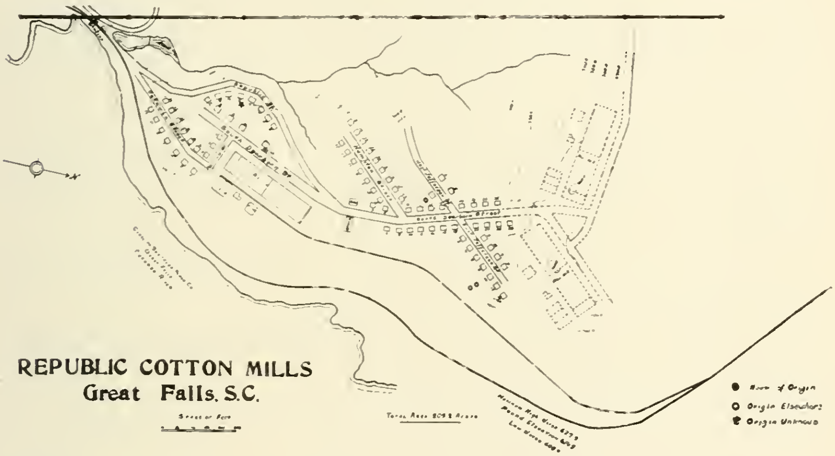

Fig. 5.—Distribution of pellagra in the Republic mill-village. Note that with one possible exception all pellagrins contracted the disease elsewhere and moved into the village with pellagra.

by the assumption that a period of three years is not sufficient time for the disease to manifest itself in a new locality.

Each of the villages here cited is still under observation, and it is our intention to follow them for at least another year, both in order to determine the presence or absence of pellagra and to ascertain, if possible, any factors other than the presence of water-carriage systems of sewage disposal which may differentiate them from those villages showing a high prevalence of pellagra.

THE SPREAD OF PELLAGRA IN UNSEWERED RURAL COMMUNITIES

We have referred to inequalities in the distribution of pellagra in Spartanburg County and have cited the fact that our records showed an incidence rate of sixteen per ten thousand of population in rural communities, against 104 per ten thousand in mill-village communities.

So far as disposal of excreta is concerned the only important difference between these two elements of the population was the fact that unscreened surface privies were always found in mill-village communities, while in rural communities, not infrequently, there was no visible method of disposal. The absence of privies applied more particularly to the colored tenant farmers. Where privies were found in rural communities they were practically always of the unscreened surface type.

The most important contrast between rural and mill-village communities appears to be density and congestion of population. There were isolated instances in rural communities in Spartanburg County where foci of pellagra appeared to exist and we made an extensive study of one such district. In this farming district covering an area 2 miles square, there was a total population of approximately 260 persons, 100 white and 160 colored. We secured records of a total of eleven cases of pellagra occurring in this population since 1906, four cases in the white population and seven cases in the colored. The incidence rate for pellagra in this rural community was 423 per ten thousand of population while the incidence rate for the rural population of the county as a whole was only 16 per ten thousand of population. In this district a history of close association between previously existing and new cases was demonstrable. There was a general tendency for active foci of pellagra in rural communities to occur in townships in which mill-villages were located and the focus mentioned above occurred within about 2 miles of a mill-village community where pellagra was endemic.

The methods of disposal of human wastes in the county in which our studies were undertaken are typical of those in use throughout the southern states where pellagra prevails so extensively. Recent statistical reports of the Rockefeller Sanitary Commission[1] indicate quite clearly the sanitary conditions existing in the Southern states at the present time. Of 189,510 homes inspected in rural communities it was found that 95,735 had no privy. The sanitary index for the total number investigated was only 5.8 per cent.

THE PREVALENCE OF PELLAGRA IN INSTITUTIONS WITH WATER-CARRIAGE METHODS OF DISPOSAL OF SEWAGE

The prevalence of pellagra in institutions for the insane, having water-carriage systems of disposal of sewage, is of particular importance in the discussion of any possible relationship between the spread of pellagra and methods of disposal of human excreta. We have been unable to obtain any definite information relative to the actual incidence rate of pellagra in asylums in the southern states. The disease is endemic in these states and cases of pellagrous insanity are being admitted to these institutions at frequent intervals. Very frequently symptoms of the disease are observed within a short time after admission and even though there be no suspicion or definite evidence of the disease on admission, it is impossible, from the histories obtainable, to arrive at any definite conclusion as to where the person contracted pellagra. It is a fact, however, that, not infrequently, patients show active symptoms of the disease for the first time, several years after commitment.

So far as we have been able to ascertain, there is probably only one institution for the insane in this country in which a severe epidemic of pellagra has occurred and in which there appeared to be no question that practically all the patients contracted the disease in the institution. We refer to an epidemic of pellagra at the Peoria State Hospital at Peoria, Ill.[2] Two of the members of this commission took part in an investigation of this epidemic and it was studied most thoroughly by the Pellagra Commission of the State of Illinois. Dr. George A. Zeller, superintendent of this institution, recalls very distinctly that cases of pellagra occurred in the Peoria Hospital as early as 1907 and has records of such cases, though they were not, at that time, recognized as such. In 1909, however, there was a sharp outbreak of pellagra in the institution involving more than 8 per cent. of the inmates (177 cases). Table 1 shows the total number of incident cases for each year from 1909 to 1913 inclusive. All cases for 1909 are tabulated as incident cases because accurate records were not available prior to that time. The mortality rates in this epidemic were very high, particularly so during the year 1909 (50 per cent.). The severe outbreak of pellagra in 1909, with the sharp decline in succeeding years, is most interesting.

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[1] Fourth Annual Report, The Rockefeller Sanitary Commission.

[2] Report of the Pellagra Commission of the State of Illinois, November, 1911, Springfield, Ill., 1912.

TABLE 1.—Incident Cases of Pellagra at the Peoria State Hospital, Peoria, Ill.

Year	1909	1910	1911	1912	1913	1914 (to March 15)
Cases	177	67	14	0	0	0

The institution had a most excellent water-carriage system of disposal of sewage and it is not possible to attribute the 1909 epidemic to improper methods of disposal of human excreta. It is to be remembered, however, that in many infectious diseases of intestinal origin, dependent mainly for their spread on faulty methods of disposal of human excreta, intimate association and contact may also be important factors. With this in mind, there are some suggestive facts which may be cited in connection with this epidemic.

TABLE 2.—Pellagra Arising in the Wards of the Peoria State Hospital

Ward	Type of Patient	Habits*	Sex †	Population	No. of Cases of Pellagra	Incidence Per Cent.
Infirmary *A*	Infirm chronic	±	♀	60	10	16.6
1 *A*	Staff house	....	....	....	....	....
2 *A*	Chronic	±	♀	50	7	14.0
3 *A*	Demented	—	♀	62	19	30.6
4 *A*	Epileptic	+	♀	60	7	11.7
5 *A*	Acute and chronic	+	♀	52	2	4.0
Infirmary *B*	Infirm chronic	±	♂	60	3	5.0
1 *B*	Acute and chronic	+	♂	52	2	4.0
2 *B*	Demented	—	♂	52	13	25.0
3 *B*	Demented	—	♂	62	4	6.4
4 *B*	Epileptic	+	♂	58	8	13.8
1 *C*	Demented workers	+	♂	110	8	7.3
2 *C*	Demented	±	♂	110	9	8.2
3 *C*	Demented	±	♂	110	8	7.3
4 *C*	Irritable dements	+	♂	110	7	6.4
5 *C*	Irritable dements	±	♀	110	14	12.8
6 *C*	Irritable dements	+	♀	110	7	6.4
7 *C*	Feeble dements	±	♀	110	6	5.5
8 *C*	Infirm old	+	♀	110	23	20.8
South dormitory	Quiet workers	+	♀	...	2	
Tent Col. *B*	Tuberculous	±	♀	57	12	21.0
Tent Col. *C*	Tuberculous	±	♂	57	5	8.8
Supply ward	Workers	+	♂	140	4	2.9
Domestic ward	Workers	+	♀	64	1	1.5

* The sign + indicates that patients are all cleanly in habits: ± indicates that some are cleanly but many are not; — indicates that all patients are uncleanly. In this column ♂ stands for male and ♀ for female.

The wards of this institution were constructed on the pavilion plan. Twelve of the pavilions had capacity for 60 patients. Eight pavilions housed 110 patients each; the supply ward had 140 patients and the domestic ward, 64. In assigning patients to wards an effort was made to distribute them according to cleanliness of habits. Through the courtesy of Dr. H. D. Singer, director of the Illinois Psychopathic Institute, Dr. George A. Zeller, member of the State Board of Administration and Dr. E. Z. Levitin of the Medical Staff of the Peoria State Hospital, we have secured information[3] covering the distribution of 181 cases of pellagra arising in the early stage of the epidemic at Peoria. Table 2 shows the distribution of cases in the different wards and each case has been charted in the ward of apparent origin.

It will be noted from this table that, except for Wards 8 *C* and 3 *B,* there was a distinctly greater tendency for pellagra to spread in wards housing untidy patients (3 *A* and 2 *B*) than in those housing patients of cleanly habits. It is also evident that the patients in 8 *C,* which shows a high rate of incidence, were of advanced age and infirm,

Omitting the two cases which occurred in the south dormitory, for which population figures are not at hand, there remain 179 pellagrins. The distribution of these cases according to cleanliness of habits is shown in Table 3.

TABLE 3.—Distribution of Pellagra in Wards of the Peoria State Hospital According to Cleanliness of Habits

Character of Inmates	Population	No. of Pellagrins	Incidence Per Cent.
All cleanly habits	866	69	8.0
Mixed: some cleanly habits and many uncleanly	724	74	10.2
All uncleanly habits	176	36	20.5
Total	1,766	179	10.1

The incidence rate of pellagra in the untidy wards was 20.5 per cent., two and a half times greater than in wards housing patients with cleanly habits, in which it was 8 per cent.

The food for the inmates was distributed from a central kitchen. One of the early and severe cases in the institution was that of a woman employed in the central kitchen. She had a very severe attack of pellagra during the summer of 1908 while working in the kitchen and her symptoms became so severe as to necessitate her admission to the hospital.

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[3] Personal communication, March, 1914.

In August, 1909, Dr. Zeller, the superintendent of the institution, decided to establish in the hospital proper, male and female pellagra wards. In the fall of 1909 practically all the pellagrins in the institution were transferred to these wards, a graduate nurse was assigned to each ward and daily notes were made on the cases. During the summer of 1910, the pellagrins were still segregated and as new cases of the disease arose they were transferred to hospital. Dr. E. Z. Levitin of the hospital staff informed us recently (March, 1914)[4] that segregation was practiced until the fall of 1911, when the patients were so few that the practice was discontinued. He states further that, except for one chronic pellagrin, there are at the present time (March, 1914) no active cases of pellagra in the institution. This segregation and practical isolation of pellagrins may possibly have been a factor in the remarkably rapid disappearance of pellagra from this institution. The death-rate among pellagrins in this institution was also remarkably high, thus diminishing the number of hypothetical sources of infection.

DISCUSSION

This study shows inequalities in the geographical distribution of pellagra in Spartanburg County. Furthermore, in those communities and elements of the population in which methods of disposal of human excreta were similar, its distribution was still unequal. In the section in which our studies were undertaken, congestion of population, general sanitary conditions being equal, appeared to be an important factor in producing higher incidence rates. Thus we found that, where the population was congested in Spartanburg County, as was the case in mill-village communities, the pellagra morbidity per ten thousand of population was six and a half times greater than in rural communities where the population was sparse. (Rural rate per ten thousand was 16, mill-village rate per ten thousand was 104.)

In the city of Spartanburg there was a distinct tendency for the endemic foci of the disease to be confined to those sections of the city in which unscreened surface privies were in use. Information given us by physicians practicing elsewhere in the South indicates that the distribution of the disease, in at least some other southern cities, coincides, so far as methods of sewage disposal are concerned, with its distribution in the city of Spartanburg. It is our intention, during 1914, to study the distribution of the disease in a number of cities and industrial communities, with particular reference to methods of disposal of sewage.

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[4] Personal communication.

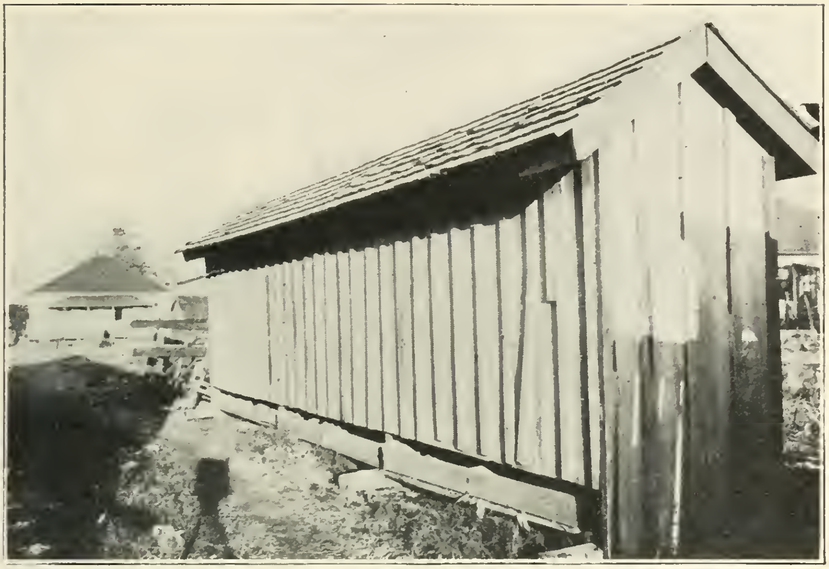

Fig. 6.—Type of unscreened trough privy formerly in use in Spartan mill-village.

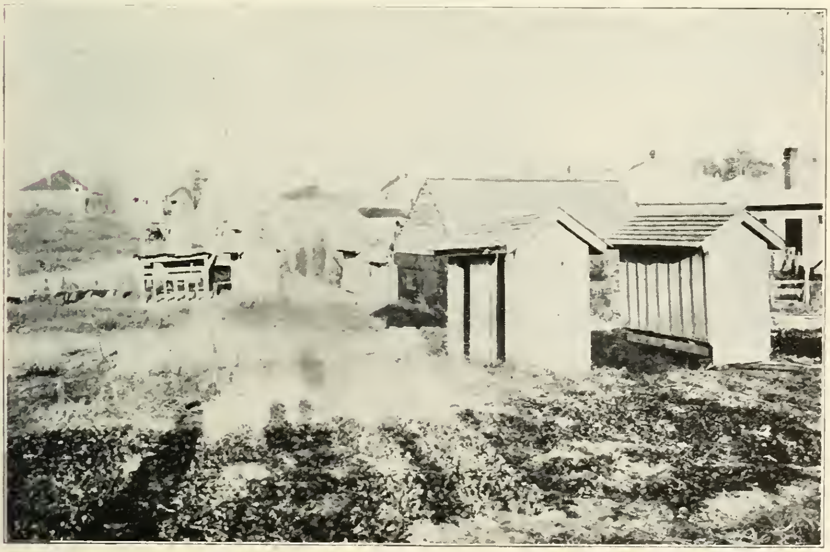

Fig. 7.—General view of unscreened trough privies formerly in use in Spartan mill-village.

Pellagra prevailed and originated in all the mill-village communities in Spartanburg County and in all these communities the methods of disposal of excreta were unscreened surface privies. We investigated the history of pellagra in two mill villages elsewhere in the state in which the only striking contrast was the fact that a complete water-carriage system of disposal of sewage had been installed. In these villages we found that, notwithstanding the fact that pellagrins moved in from time to time, thus far, there had been no tendency for pellagra to originate or to spread in these communities. These observations appear to us to be somewhat striking and strongly suggest the possibility of a direct relationship between the spread of pellagra and faulty methods of disposal of human excreta.

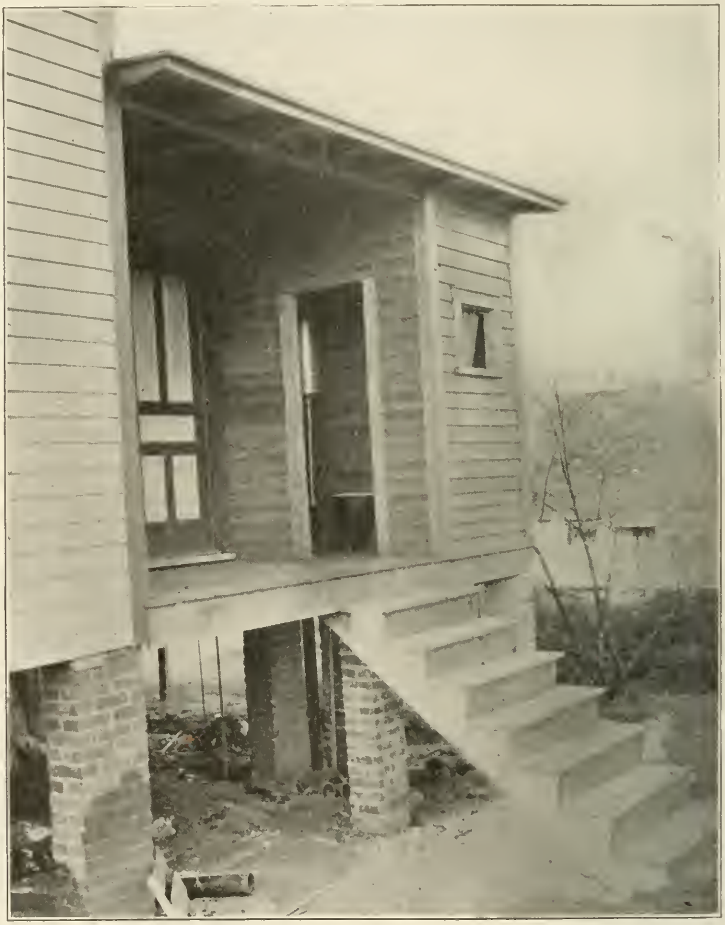

Fig. 8.—Type of water-closet recently installed in Spartan mill-village.

There is a wide-spread movement under way at the present time in the southern states toward the improvement of methods of disposal of human excreta. Many towns and cities as well as industrial communities in which pellagra is endemic are either improving their types of surface privies or putting in modern water-carriage systems of disposal. It is in such communities that most important information can be obtained, either confirming or refuting these observations. Superficial studies and general impressions will not suffice. If any information of value is to result, the local health officers and physicians in communities, in which improved methods of disposal are being installed, must necessarily know the history of the prevalence and distribution of pellagra prior to the installation of improved methods.

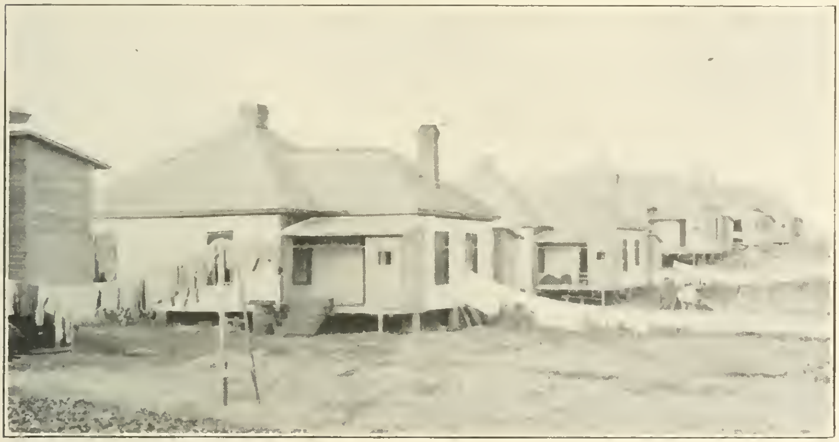

Fig. 9.—Rear view of a row of houses showing type of construction adopted in installation of water-carriage system of disposal of sewage in Spartan mill-village. The closet is on the back porch.

They must also necessarily keep accurate records, for at least three or four years, of the prevalence of the disease subsequent to the installation of improved sanitary methods.

One community exists in Spartanburg in which a water-carriage system of disposal has been substituted for unscreened surface privies. Reference to Figure 2 (the distribution of pellagra in the city of Spartanburg and its relation to methods of disposal of sewage) shows that the disease has prevailed most extensively in the northwestern quadrant of the city. In this quadrant is located one of the largest cotton-mills in the county. About 300 of the dwelling houses in this section are the property of this mill, and during the summer of 1913 the president of the mill, Mr. W. S. Montgomery, decided to install individual water-closets in each dwelling. Up to the present, pellagra has been excessively prevalent in this section and the method of disposal of human wastes has been the unscreened trough system (Figs 6 and 7). The installation of a water-carriage system was begun in the fall of 1913 and is being pushed to completion. On Feb. 1, 1914, 123 installations had been completed and connected with the sewer mains. The type of construction adopted is shown in Figures 8 and 9. A special non-freezing type of closet has been installed, as the fixtures are exposed to sudden and sharp changes in temperature.

We have studied the prevalence of pellagra in this village during 1912 and 1913 and each case has been carefully located. We know that at least eighty cases of pellagra have originated in the northwestern section of the city during the past six years. It is our intention to observe carefully the behavior of the disease with respect to the development of new cases during 1914 and for two or three years thereafter.

In presenting these facts at this time, we hope that similar studies may be undertaken in communities elsewhere in the Southern states where pellagra is endemic and where contrasts in methods of disposal of human excreta present themselves.

SUMMARY

1. Pellagra morbidity was higher in congested communities using surface privies than in more sparsely settled districts in which similar methods for the disposal of excreta were employed.

2. In the city of Spartanburg the endemic foci of pellagra were located in the districts in which surface privies were in use.

3. In cotton-mill villages equipped with surface privies pellagra was found to be endemic and new cases of the disease arose there year after year.

4. In two cotton-mill villages completely equipped with water-carriage systems of sewage disposal it was impossible to find cases of pellagra which had certainly originated there, although some cases which had originated elsewhere were present.

5. There is some evidence that pellagra spreads in hospitals for the insane more readily in the wards housing untidy patients.

6. This study indicates that methods of disposal of human wastes may prove to be a determining factor in the spread of pellagra in certain communities and it suggests a possible method of prophylaxis, which is now being tested in a practical way.

Twentieth Street and Second Avenue.

NOTES ON A NORMAL, PRESYSTOLIC SOUND *

E. W. BRIDGMAN, M.D.
BALTIMORE

In the course of some work recently undertaken in Dr. Thayer's service to localize the third heart-sound by the use of simultaneous tracings, a remarkably constant, presystolic sound-representation was observed on the electrophonogram; and its description will be the effort of this paper. The technic employed, the value of the Einthoven electrophonograph, and some of the factors concerned in the audibility of cardiac sounds have been previously discussed;[1] but attention may be recalled to the constancy and reliability of apex-tracings obtained in the left decubitus, and to the localization of the first sound by the foot of the ventricular rise of the apex cardiogram.

In a reconsideration of the tracings of sixteen normal schoolboys, it may be said that the pulse-rate varied from 65 to 100, diastasis[2] ranged from 0.12 to 0.50 second in the series, and from 0.21 to 0.39 second in an individual case, as opposed to a fairly constant systole and diastole, not only for the individual, but for the series.

Eleven of the apex-tracings showed definite auricular waves; these were in no way to be confounded with the wavelet sometimes seen on the anacrotic limb of the apex-tracing that marks the presphygmic period — the time between the onset of ventricular systole and the expulsion of blood into the aorta. The distance from the beginning and summit of the auricular wave is shown for the individual cases elsewhere (see Table); here it is sufficient to say that the beginnings of ventricular and auricular systole are separated by about 0.09 of a second, while the summit of the elevation associated with auricular systole follows its onset by closely 0.04 of a second.

Eleven of the phonograms showed a short presystolic sound-wave. Three of these were in cases in which no auricular waves were shown, while three boys, with auricular representation on the apex curves, showed no presystolic wave on their phonograms. This lack of agreement speaks strongly against simple pressure phenomena as being responsible for the phonographic curves. The presystolic sound begins, on an average, 0.07 of a second before the appearance of the first sound and at varying intervals after the preceding third and second sounds, depending on the inconstant diastasis. It is repre-

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* Submitted for publication, June 10, 1914.

[1] In a communication to appear shortly in Heart.

[2] Henderson, Yandell: Am. Jour. Physiol., 1906, xvi, 325.

sented by a wave which, in most instances, shows displacement in but one direction and is probably, therefore, exposed to marked damping at the source. The wave is of a duration of closely 0.04 of a second, making the sound of a frequency, roughly, in the vicinity of 25 vibrations per second; and thus, in these cases, below the limits of human audibility. The amplitude averages about one-eighth the amplitude of the first sound, though it varies from one-fifteenth to one-fourth. In some cases, its corresponding galvanometric oscillation was greater than that of the third sound, which in several cases was clearly marked out in the same cardiac cycle. On the phonograms taken to show the greater amplitude of the third sound following increased venous pressure, as after elevation of the limbs, moderate exercise, etc., the presystolic sound shows similar accentuation.

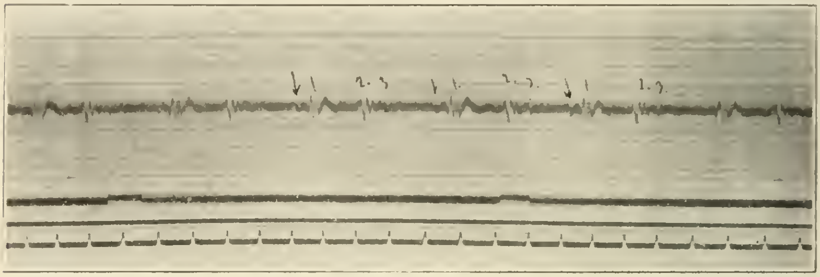

Fig. 1.—Electrophonogram of C. C., showing a definite presystolic and third sound. This and the following electrophonograms were taken with patient lying on back, and with varying strengths of primary circuit.

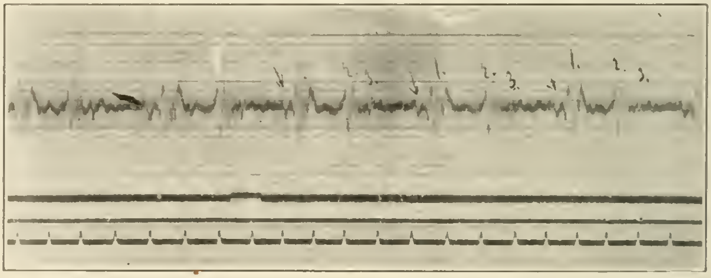

Fig. 2.—Electrophonogram of C. C., showing definite presystolic and third sounds. Arrows point to presystolic sounds.

Attention has been called to the danger of drawing absolute conclusions from these phonographic waves; they do not represent true sound-waves, but their transformation into electrical energy. It seems fair, however, to make these comparative statements which afford a mental concept as to the duration, position, and pitch of this sound.

There is, then, in many normal boys a presystolic sound that is generally below the limits of human audibility, and which occurs about 0.02 of a second after the beginning of auricular contraction. Further description would involve the introduction of pathologic cases and would necessitate a discussion of the presystolic gallop—factors not to be undertaken in this description of a normal phenomenon.

It is interesting to endeavor to put forth some hypothesis as to the etiology of this sound—a hypothesis which, of course, must be substantiated by the known facts, and which must also be adaptable to pathologic conditions. In a normal cardiac cycle, the ventricle is nearly empty of blood at the end of systole; in 0.03 of a second after the closure of the aortic and pulmonic valves, the auriculoventricular valves open to allow the active period of ventricular filling—diastole. During this time, blood flows into the ventricle to an amount and to a resultant ventricular tension, depending first on the pressure of the blood dammed back into the auricles and veins by the preceding systole, secondly, on the tone of the ventricle, and thirdly on outside factors. Of outside factors, gravity is an example; for with the patient on his left side more blood is going to flow in because the tonicity of the ventricles must withstand an additional factor besides venous pressure —the force of gravity. The blood, then, flows in until there is a definite tension of the ventricular walls—a condition which is associated with the approximation of the auriculoventricular valves. The

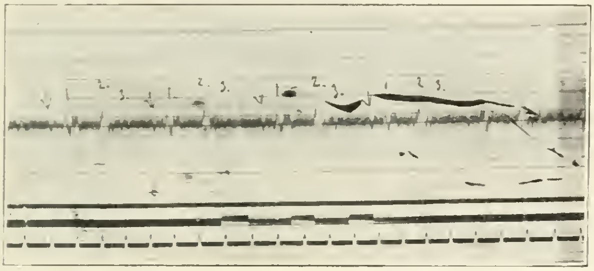

Fig. 3.—Electrophonogram of C. P., showing definite presystolic and third sound. Arrow points to presystolic sound.

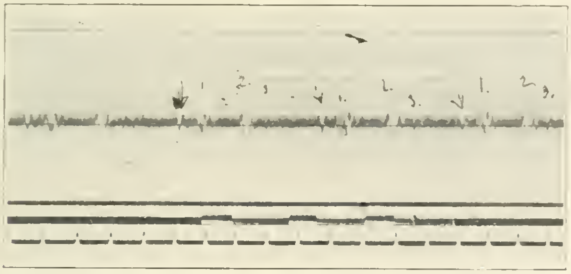

Fig. 4.—Electrophonogram of C. P., showing well-marked presystolic sound with a definite third.

ventricles are pretty well shut off from the auricles unless there is a very high, and rapidly increasing venous pressure, when the ventricles may be still further slowly distended by a small amount of blood oozing through the auriculoventricular apertures. As the ventricles rapidly distend, they may suddenly become taut, because of their tone, and there would, of necessity, be vibrations of the walls and thereby sound production. Were the ventricles distended with air a louder sound would be expected, but marked damping occurs with the particular type of fluid contact. With the onset of auricular contraction, more blood is thrown into the ventricles, whose walls become correspondingly more taut. At the same time, the whole floor of the auricles strikes against the distended, elastic bag, producing conditions favorable for sound formation. With the onset of systole, tension of the papillary system and the contractions of the ventricular walls on the contained fluid might produce vibrations of the stretched tissue. During systole, as the ventricles contract steadily, the sudden impulse would be lost and the tissue itself would be shortened. So that the conditions favorable for sound would appear only in very early systole. At the end of systole there is no stretch of the walls —they are relaxed—so until they are made taut by blood distention there would be no expectation of sound production. This conception of muscle vibration has nothing to do with the valvular elements of the heart-sounds, elements which are so definitely the most important factors in their production. But it might explain the so-called "muscular element" of the first sound which is so hard to understand. The possibility of the vibration of a stretched and suspended elastic substance when acted on by an outside force offers a hypothetical explanation of the presystolic sound. That such vibrations may be associated with the valvular element of the first and third sound has suggested experimental investigation that is being undertaken.

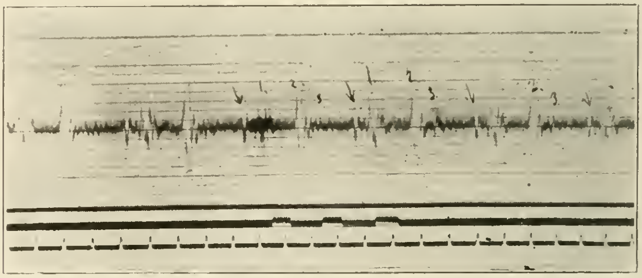

Fig. 5.—Electrophonogram of C. P., showing an unusually definite presystolic sound with third sound. Arrow points to presystolic sound.

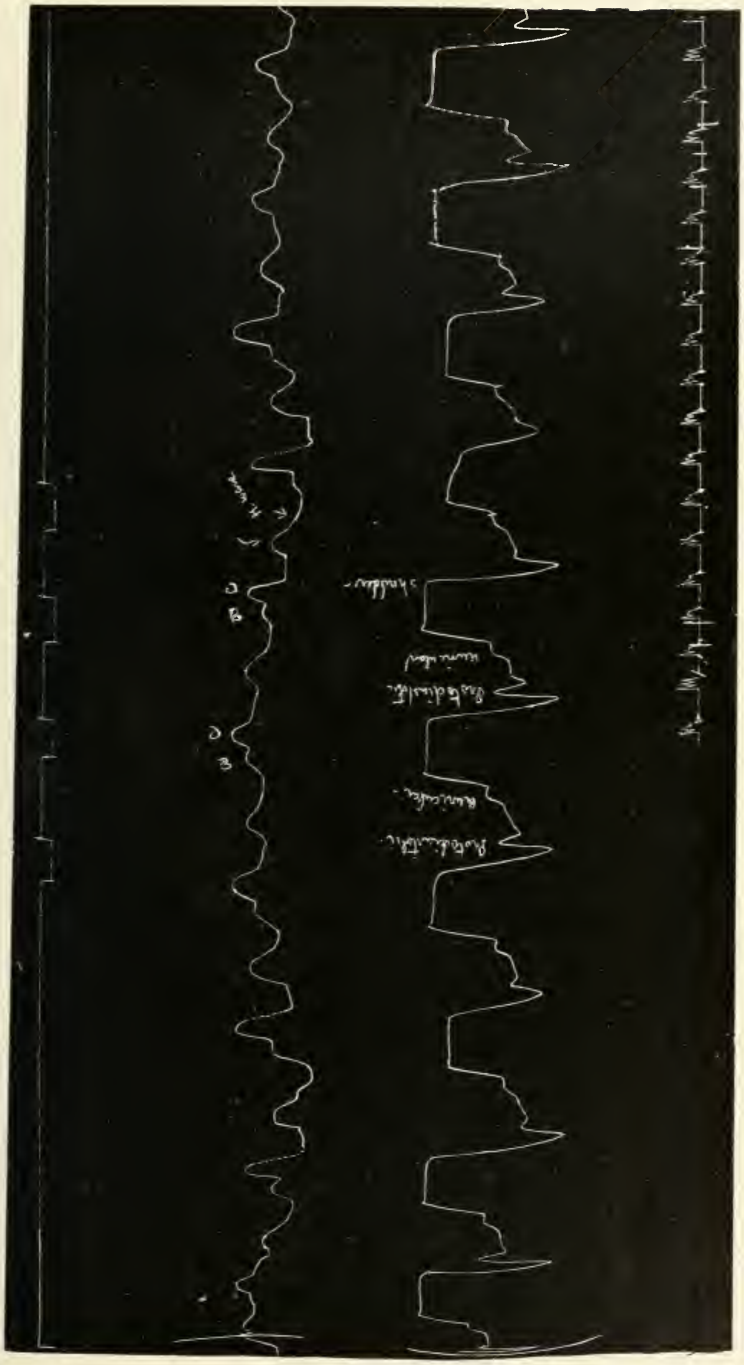

Fig. 6.—Simultaneous apex and venous tracing with auricular and protodiastolic waves on apex tracing.

Effort has been made rather successfully to correlate the conception of the presystolic sound with different pathologic conditions, and to suggest its connection with the presystolic gallop. The sound and shock appear here to be but the accentuation of the normal presystolic sound with its frequently associated presystolic wave on the apex cardiogram. This appears to substantiate the view of Prof. Friedrich Müller that the different gallops represent pathologic exaggerations of events which take place in the normal heart. On searching the literature, it was found that a presystolic sound or "grace note" has been already described by von Wyss, who employs the same technic. His time-results compare very closely with those set forth in the subsequent table.

COMPARISON OF THE ELECTROPHONOGRAMS AND APEX-TRACINGS*

Name	Presystolic Before First	Beginning of Auricular to Beginning of Ventricular Wave
E. W.	0.07 sec.	No auricular
L. G.	No presystolic	No auricular
J. B.	No presystolic	No auricular
C. P.	0.09 sec.	0.12 sec.
J. S.	0.06 sec.	0.08 sec.
E. G.	0.10 sec.	0.10 sec.
C. C.	0.07 sec.	0.06 sec.
B. W.	No presystolic	0.11 sec.
R. T.	0.06 sec.	No auricular
N. B.	No presystolic	0.10 sec.
W. N.	0.08 sec.	0.08 sec.
I. R.	0.06 sec.	0.08 sec.
B. R.	0.08 sec.	0.09 sec.
A. S.	0.07 sec.	No auricular
A. L.	0.06 sec.	0.08 sec.
N. S.	No presystolic	0.08 sec.

* These measurements represent the average of ten sequential beats.

SUMMARY

A presystolic sound, which is generally below the limits of human audibility, is shown on the phonographic tracings of a number of normal boys.

It occurs 0.02 second after the beginning of the auricular wave of the apex cardiogram, 0.07 second before the beginning of the first sound; is of a duration of 0.04 second.

A possible etiologic factor is suggested, the tension of the ventricular walls, and a possible relationship between this sound and the presystolic gallop is called to mind.

It is a pleasure to thank Dr. George Bond of Johns Hopkins Hospital for his interest and assistance in obtaining the tracings.

PERIARTERITIS NODOSA — A CLINICAL AND PATHOLOGICAL REVIEW OF THE DISEASE

WITH A REPORT OF TWO CASES *

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NEW YORK

INTRODUCTION

A number of diseases which are to-day not uncommon and which are recognized clinically without much difficulty were at one time quite rare, owing largely to the fact that so little was known about them that they either escaped notice or were incorrectly diagnosed. It is quite possible that the rather rare but exceedingly interesting affection known as periarteritis nodosa is a case in point. Most of the cases have been observed in Germany, where the disease was first recognized and where the clinicians and pathologists have come to be familiar with it, whereas in this country, where the disease has attracted very little attention, only three cases have been reported up to the present time.[1]

Within the past year at the Presbyterian Hospital, we have had the exceptional opportunity of seeing two cases. Including these, the total number of reported cases now numbers but thirty-eight. Most of the reports have concerned themselves largely with the pathologic histology of the disease and, except for some minor differences of opinion, this aspect of the affection has been well covered. We are still quite in the dark as to the cause, however, and many of the histories are too brief to aid greatly in arriving at a correct idea of the symptom-complex. Indeed, many observers are inclined to the belief that, barring the presence of the subcutaneous nodules, which are present in only a small minority of the cases, there is no set of symptoms on which a diagnosis can be made. One cannot escape the conviction, however, that an increasing familiarity with the disease may lead to some means of diagnosis whereby more cases may be recognized and that the chance for study during life thus afforded may clear up the present obscure etiology. It, therefore, seems most desirable that all cases of periarteritis nodosa be reported as completely as possible. And, in view of the extreme rarity of the disease in this country, the present seems an opportune time for a complete review of the known facts concerning it.

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* Submitted for publication, June 1, 1914.

* From the Pathological Department, Presbyterian Hospital, Columbia University, New York.

[1] The two cases here given were very briefly reported at the New York Pathological Society during the past year.

REPORT OF TWO CASES

For permission to use the clinical records of these two cases I am greatly indebted to Drs. Janeway, Bovaird and Lambert on whose services in the hospital they occurred.

CASE 1.—Man, aged 26, mechanic, ill about seven weeks, was admitted to the hospital, Dec. 7, 1912. The family history was unimportant.

Past History.—The patient had typhoid at the age of 4. Two years ago he had an attack of grip. Venereal disease is denied. He does not use tobacco; takes an occasional glass of beer.

Present History.—For the past two years patient has had asthma. At first the attacks came about once a month, lately as often as two or three in a week. About three weeks before admission he noticed intense itching about the right ankle and in eight or ten hours an oval, node-like swelling the size of an egg appeared. The overlying skin was tense, shiny and covered with bright red macules measuring about 4 mm. in diameter. The swelling gradually lessened and the macules underwent the color graduations of a contusion. In two or three days the swelling and eruption had entirely disappeared. Pain and tenderness were severe at the height of the swelling, disappearing with its subsidence. The night after the swelling appeared he had a severe asthmatic attack lasting all night. Expectoration was quite profuse and contained clotted blood. Since then he has had a purpuric eruption appearing on different parts of the body roughly every other night with asthmatic attacks on the alternate nights. All parts of his body have been affected except the face. For the past two weeks, accompanying the rash, he has had severe abdominal cramps lasting for ten or twelve hours and ceasing after vomiting. Vomitus consisted of a thin green liquid with a few blood-clots. At times, with the appearance of the eruption he would have pains in various joints and these would be swollen, slightly red, tender and very painful on any motion. Bowels moved once or twice a day and were normal as far as he knows. Appetite is good; no loss of weight; no fever or night-sweats. For the past two weeks patient has been up ten or twelve times at nights to urinate.

Physical Examination.—Pale and sallow. Superficial lymph-nodes slightly enlarged. Eyes normal. Gums firm. No throat lesions. Lungs: Moderate relative dulness at right apex posteriorly; feeble inspiratory murmur with prolonged expiration and high-pitched piping râles over all. Heart: Apex in fifth space, 10.5 cm. to left of mid line; sounds normal, no murmurs. Arteries small, soft and just palpable. Blood-pressure 145-105. Abdomen rather full and tympanitic; liver not felt; edge of spleen just felt at costal margin on deep inspiration; no masses or rigidity. Both legs show numerous small hemorrhagic areas in various stages of involution. These areas vary in size from petechiae to irregular confluent areas about 1 cm. in length. The larger ones are grouped about the ankles and are deep wine color. The lesions are somewhat raised, firm and dry with a tendency to scaling. On the left thigh, just above the knee, there is a large, somewhat elevated, firm area the size of the last joint of the thumb. It is pale green, somewhat tender and apparently in the course of the internal saphenous vein but not connected with it. On the right thigh there is an elongated, raised area measuring 3 by 1 cm. It is reddish brown with dark red spots in the center, is very slightly tender and for its whole length lies over a superficial vein. About the knees there are a few pale green discolorations. Over the lower end of the right tibia there is an irregular depressed ulcer. It is dry and the floor is made up of a deep red blood-clot. Below the left patella there is some thickening in the region of the bursa, with slight pain on flexion of the leg. The most probable diagnosis seemed to be Schönlein's "purpura rheumatica."

Course.—The temperature on admission was 100.4 F., gradually falling to normal on the fifth day. It remained normal for three days and then rose gradually to 101.8. In a few days it was again normal and then subnormal for five days. There was another short rise to 100.4, followed by another normal period and a terminal rise to 102. The pulse varied between 85 and 130 and was generally more rapid than one would expect for the temperature. When the temperature was subnormal, the pulse was between 80 and 110.

Urine.—Eight examinations. The specific gravity varied between 1.010 and 1.018. There was always a trace of albumin present and on five occasions it was estimated at from 0.15 to 0.22 per cent. No sugar was found. There were always hyaline and granular casts present and at times epithelial and waxy casts as well; also a moderate number of leukocytes and a few to many red blood-cells.

The blood-counts showed the following result:

Date	R. B. C. No.	Hb. Pct.	W. B. C. No.	Polys. Pct.	Lymphos. Pct.	L. Monos. Pct.	Eosin. Pct.
December 9	....	75	17,200	50	14	2	34
December 10	3,500,000	65	20,200	51	11	0	38
December 12	....	..	......	32	12	5	51
December 31	3,384,000	42	20,900	58	17	2	23

December 9: Sputum shows many red blood-cells and ciliated epithelial cells but no Charcot-Leyden crystals, Laennec's pearls or Curschmann's spirals.

December 12: Eye-grounds negative.

December 14: Wassermann reaction (Noguchi modification) negative.

December 17: Since yesterday afternoon patient has complained of constant, sharp abdominal pain without any other change in the physical signs. No vomiting. Stools normal. No tubercle bacilli found in the sputum.

December 18: Phthalein test: Color appeared in twelve minutes. Amount at the end of two hours too small to estimate. Sputum bloody. Coughs less. Feels better. Abdominal pain has disappeared. Lips and nails quite cyanotic. No tubercle bacilli in sputum.

December 20: Slight pain in the shoulders and neck this morning. Has a considerable amount of yellowish-green sputum with considerable fresh blood. The lungs show nothing more than the signs of asthma and bronchitis. No pain in the chest. Considerable relief from epinephrin.

December 29: White, rather glistening lines are seen along the vessels of the left retina as they come through the disk.

December 31: Gastric contents: Total acidity, 38. Free hydrochloric acid, 6. No lactic acid. Moderate reaction for blood. No ova or parasites in the stool. A second phthalein test showed the color in seventeen minutes, but the amount at the end of two hours was again too small to estimate.

January 1: The veins and glands in both inguinal regions are very sore and tender. In the left femoral region there is a small, red, tender area.

January 2: There is more redness and swelling in the left femoral region. On the back of the left leg there is a slightly swollen, reddened area measuring about 4 by 6 cm. The right hypochondrium is distinctly tender and resistant.

Jnuary 5: There is a pericardial friction-rub present this morning and a pleural friction-rub at the left base. Lumbar puncture: Twenty c.c. of clear fluid obtained. Cultures sterile. Urea, 0.323 per cent. Sodium chlorid, 0.812.

January 6: Died to-day after increasing stupor for two days. Gradual failure of pulse and respiration before death. During the last sixty hours voided only 1,000 c.c. of urine. The blood-culture taken in two-broth flasks, January 3, remained sterile. Blood-pressure varied between 135 and 165. The urine quantity varied between 690 and 1,920 c.c. On thirteen days it was below 1,000 c.c.

Post-Mortem Examination.—The diagnosis of periarteritis nodosa was not suspected at the time of the necropsy. When the microscopic sections were examined several days later and the characteristic lesion found in the kidney, the organs from the patient were again carefully examined; but the only suggestion of arterial lesions was found in the heart, where there were one or two pin-head-sized whitish nodules on one of the small coronary branches. No nodules were found in any of the other organs. Unfortunately, none of the subcutaneous tissue in the regions of the eruption and swellings was examined.

The essential points in the necropsy were the following:

The body is strikingly pale and considerably emaciated. There are ecchymoses about the right knee and about both elbow-joints. The peritoneal cavity contains a small amount of cloudy yellow fluid, and the intestines show a scattered fibrinopurulent exudate. The lungs are adherent to the chest-wall and to the outer surface of the pericardium by fresh fibrinous adhesions.

Lungs: Both are edematous and show scattered areas of bronchopneumonia. The bronchi are intensely congested and contain mucopurulent material. The vessels appear normal.

Bronchial Lymph-Nodes: Moderately enlarged, deeply pigmented and firm.

Heart: The pericardium contains about 50 c.c. of cloudy yellow fluid; there are several fresh fibrinous adhesions stretching between the two surfaces and the surfaces are covered with a shaggy, fibrinopurulent exudate and show scattered petechial hemorrhages. The heart is not enlarged. There is a small, firm, irregular nodule at the base of one of the pulmonic cusps and the mitral cusps show small, irregularly nodular, reddish vegetations, the largest about the size of a small pea. The other valves and the heart chambers show no change. In general the heart-muscle is homogeneous and dull reddish-brown, but there are scattered areas of a more dirty yellow color. The main portions of the coronary arteries show no sclerosis. The aorta is smooth and elastic throughout its course.

Spleen: Measures 12 by 7 by 3 cm. There is perisplenitis. Otherwise the organ appears normal.

Liver: Measures 29 by 19 by 6 cm. There is a small area of superficial atrophy in the right lobe. On section, the liver shows no abnormal change.

Gall-Bladder: Normal. Ducts patent.

Kidneys: Left measures 12 by 7 by 3.5 cm. The capsule strips easily, exposing a smooth yellowish-pink surface with a rather diffuse mottling of yellow. On section the cortex does not average more than 5 mm. in thickness and is slightly less than this in some places. The striations are indistinct and quite distorted. The glomeruli are quite distinct as colorless dots. The pelvis and ureter are normal.

Right: Measures 11.5 by 6 by 3.5 cm. and is quite similar in all respects to the left.

Adrenals and esophagus appear normal.

Stomach: Contains a little brownish fluid and a moderate amount of mucus. There is considerable injection of the mucous membrane and a few small petechial hemorrhages.

Pancreas and Ducts: Normal.

Mesenteric Lymph-Nodes: These are enlarged, many to bean-size. Several of them are broken down and ooze creamy-yellow pus on section.

Intestines: Show nothing more than a few areas of congestion in the lower part of the ileum.

Bladder and Prostate: Normal.

Brain and Spinal Cord: Permission could not be obtained for their examination.

Microscopic Examination.—Lungs: Many sections from various parts of the lungs were examined. These show a thickened pleura, extensive pulmonary edema with congestion of the alveolar walls and swelling of the alveolar epithelium and scattered areas of hemorrhagic bronchopneumonia. There is some increase of connective tissue about the bronchi together with numerous mononuclear wandering cells and eosinophils. Scattered here and there in the alveoli are small clumps of Gram-positive cocci. The large vessels appear quite normal and in most of the sections even the smaller arteries show no change; but one does find a few small arteries both about the bronchi and in the lung-tissue proper which are not normal. The changes consist in an adventitial and periarterial infiltration with mononuclear wandering cells and eosinophils, polymorphonuclear leukocytes being almost entirely absent. The changes are generally unilateral or at least more marked on one side, and the media and intima are normal. An occasional small artery is seen, however, whose lumen is almost entirely occluded. These changes apparently represent an early stage of the lesion seen in the arteries in other organs.

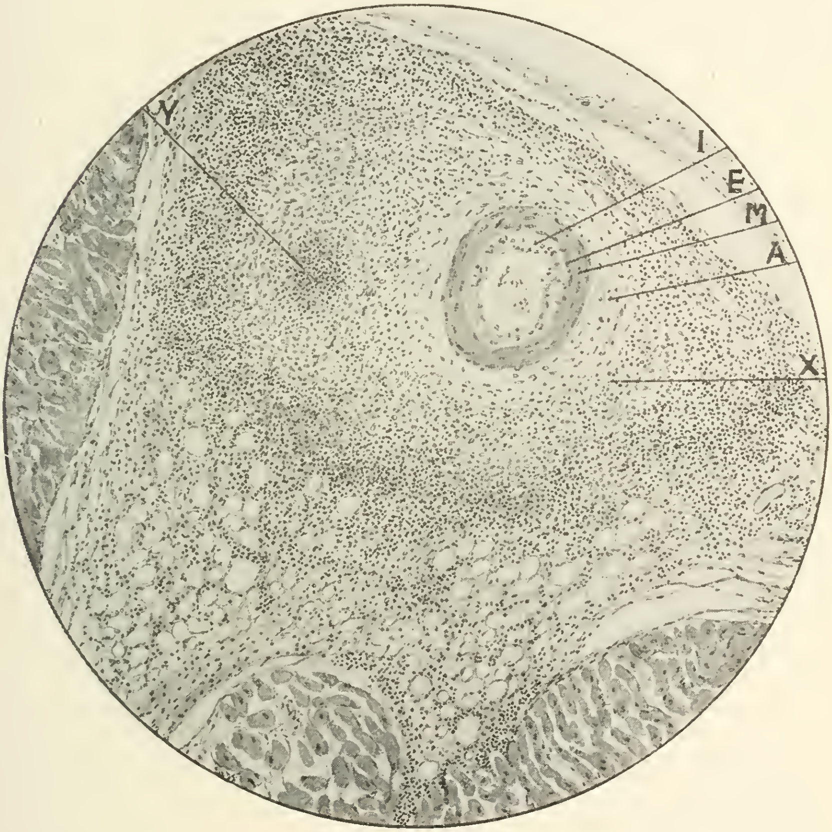

Fig. 1.—Case 1. Section of a small coronary branch just beneath the epicardium showing very pronounced periarterial infiltration with a small area of beginning necrosis on one side. The media, while still intact, takes the eosin stain rather strongly. *I,* intima; *E,* internal elastica; *M,* media; *A,* adventitia; *X,* periarterial infiltration; *Y,* area of beginning necrosis.

Bronchial Lymph-Node: In the capsule, two small arteries are found showing early changes similar to those seen in the lung. The arteries in the gland itself show no change. There is considrable pigmentation and hyperplasia.

Vegetation of Mitral Valve: Consists of a very vascular and quite cellular mass of connective tissue richly invaded by mononuclear wandering cells. On the surface is an irregular thrombus mass containing clumps of bacteria. These appear to be cocci. Smears from these vegetations show a few pairs of cocci surrounded by a small space and also some rather fat Gram-positive bacilli.

Heart: The pericardium shows an acute inflammatory exudate. There is cloudy swelling of the muscle-fibers and patchy interstitial myocarditis about the vessels. A section of one of the papillary muscles shows a most extensive fibrosis with large areas of granulation tissue and wandering-cell infiltration. Most of the vessels show no change. Some of the small arteries, while showing no changes in their coats, have a little surrounding cellular infiltration, the cells being generally mononuclear wandering cells and eosinophils. One section, taken through one of the tiny macroscopic nodules, shows the periarterial character of the lesion very well (Figure 1). It affects a small artery just beneath the pericardium. The lumen is narrowed, being encroached on by the moderately proliferated intima, and contains red blood-cells, among which are some fibroblasts extending in from the intima. The intima is thicker on one side, its texture quite loose and its cells irregular in size and shape. Some of them are quite definitely fibroblasts; others look like distorted endothelial cells. The internal elastica is well preserved except at one point, where it is wanting opposite a small necrotic area in the media. The media appears granular and the nuclei indistinct but it shows no cellular infiltration. There is no characteristic hyaline ring, but the media gives one the impressioon of approaching toward such a condition as seen in other vessels. The fibers appear more granular and take the eosin stain more intensely. The adventitia is much thickened and merges imperceptibly into the tremendous periarterial cellular infiltration and granulation tissue. The cells are wandering cells, fibroblasts and a most striking number of eosinophils. Many of them are plasma-cells. While polymorphonuclear leukocytes are scarce, one does find some. All of these cells infiltrate the surrounding fat for a considerable distance. At one side there is a small area of necrosis.

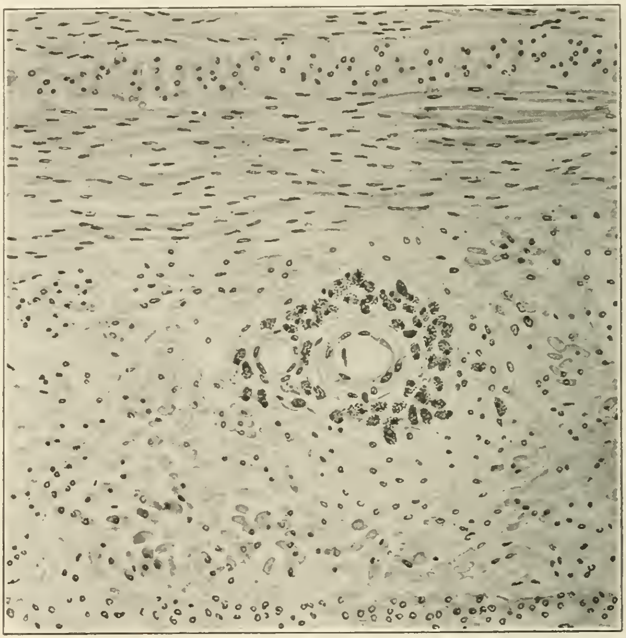

Fig. 2.—Case 1. Section of a small artery in the muscular coat of the stomach. Represents a very early lesion. The intima and media are normal. The elastic lamellae are not changed. The adventitia is loose in texture and is densely infiltrated with eosinophils.

Spleen: Eosinophils are rather strikingly numerous and seem especially attracted to the vessels. The small arteries are somewhat thickened but none of them shows any characteristic change.

Liver: Shows cloudy swelling and one small area of superficial atrophy. Some of the portal spaces show a little increase of connective tissue with a few wandering cells. There are no distinct arterial changes.

Pancreas: Shows no change except for a little wandering-cell infiltration about one or two of the small arteries. Mixed with these cells are a few eosinophils.

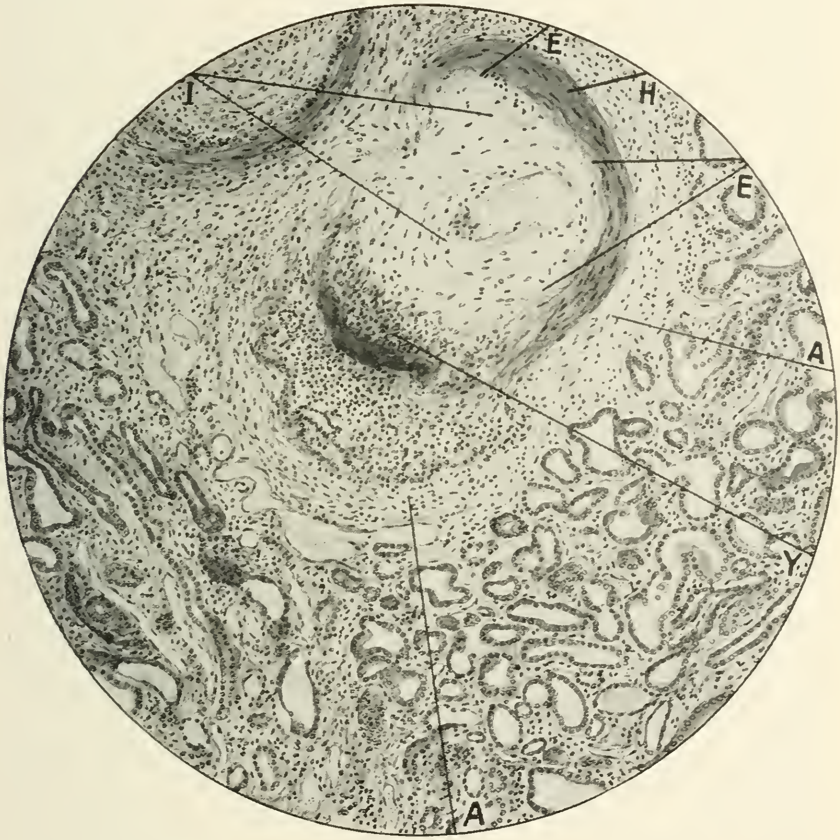

Fig. 3.—Case 1. Section of artery in kidney. The intima, *I,* is much thickened and is surrounded for two-thirds of the circumference by the internal elastic lamella which does not show in drawing. Just external to this is the hyaline ring, *H,* which fuses with the area of necrosis at *Y.* Adventitia, *A,* apparently extends outside the area of necrosis. *E,* internal elastica as shown in other sections stained with elastic-tissue stain. It is wanting opposite the area of necrosis.

Mesenteric Glands: Many small arteries are seen both within the gland and in the surrounding tissue which show early changes.

Stomach: The various coats of the organ appear normal. In one section, in the muscular coat, there is a cross-section of a small artery (Figure 2) which is of interest as it possibly shows a very early lesion. The intima and media appear normal and the elastic lamellae are unchanged. The adventitia is loose in texture and cannot be differentiated from the adjacent tissue. It is densely infiltrated with eosinophils and a few cells with large, oval, vesicular nuclei. No further change is made out in tracing the vessel through serial sections. While one cannot be certain that this represents a very early change, the eosinophils which are found so abundantly in the lesions elsewhere seem to point to this conclusion. Another section shows a typical lesion of a small artery in

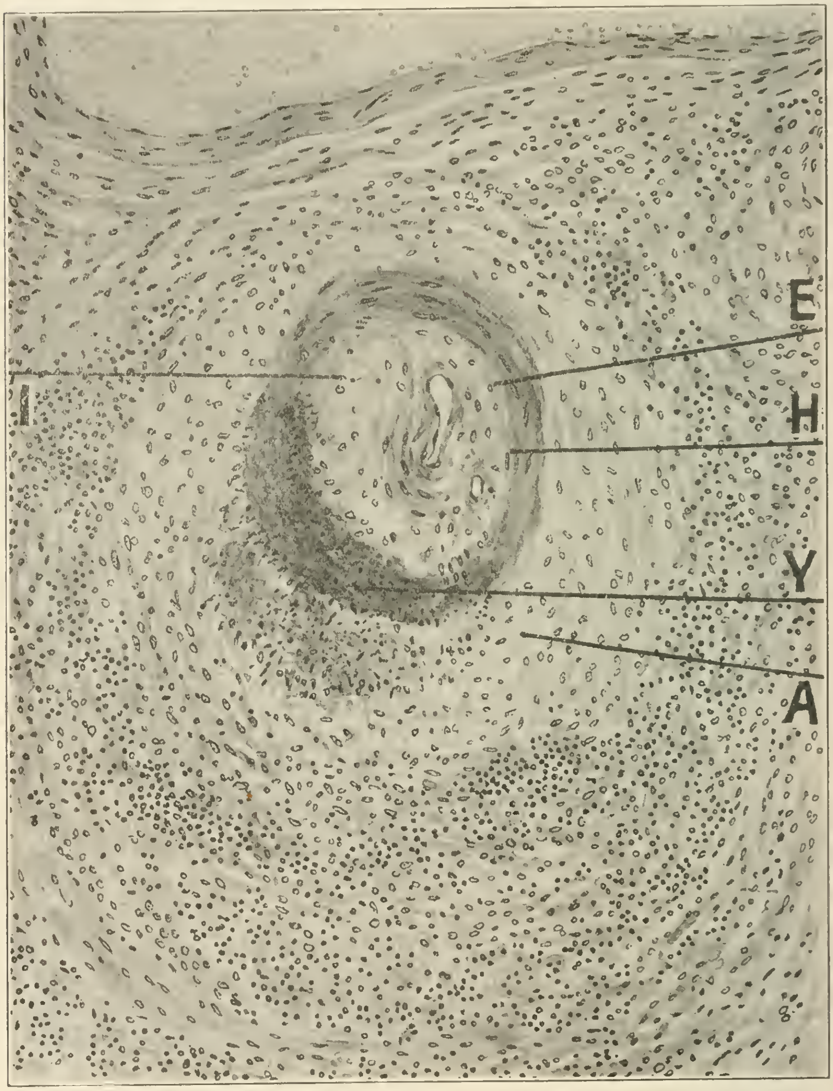

Fig. 4.—Case 1. Section of artery in adrenal. The intima, *I*, is thickened. The internal elastica, *E*, is intact except in the region of necrosis, *Y*, where it has disappeared. The hyaline ring, *H*, in which one can still make out the cells of the media lies just external to the elastica and fuses with the area of necrosis at *Y*. Adventitia, A, merges imperceptibly with the periarterial infiltration.

the submucosa. An elastic-tissue stain shows the internal elastica ending abruptly after encircling about one-half the circumference while the external elastica is completely gone. Another vessel shows similar changes but with an added complete thrombosis.

Intestines: There is an acute inflammatory peritoneal exudate, intense congestion of the vessels and some hemorrhages in the mucosa. A number of the small arteries show typical early changes and two are completely thrombosed.

Kidneys: These show the most striking changes of any of the organs, and it was here that the lesion was first noticed. In this place (Figure 3) the lumen of the artery is small and the intima very much thickened, being composed of a loose connective tissue, rich in fibroblasts and containing a few eosinophils and nuclear fragments. Surrounding the intima for about two-thirds of the circumference, the internal elastica is intact but swollen in places. Just outside of it and limited by it, is the characteristic, intensely eosin-stained, hyaline ring in which one can still make out the cells of the media. It gives the impression of being a more advanced stage of the appearance seen in the section of a coronary artery. At one side, the elastica has disappeared, the hyaline ring melts into an area of necrosis and the cellular infiltration of the adventitia breaks through into the intima. The adventitia is composed of young connective tissue with some wandering-cell infiltration and at this point seems to extend outside the area of necrosis which is mainly in the media. While this is the most advanced lesion seen in any of the many kidney-sections, many of the small arteries show similar if less marked changes. There is an exquisite acute, subacute and chronic glomerulonephritis, the glomeruli showing all stages of adherence to the capsules with epithelial crescents and hyaline thrombi. There is an extensive diffuse increase of connective tissue throughout and a striking wandering-cell infiltration with many eosinophils in the interstitial tissue. The vessels are all congested and many of the tubules contain blood. Some of the tubules are practically obliterated while some are dilated and cyst-like.

Adrenals: The arteries of the capsule show the lesion beautifully, the hyaline ring being especially well marked (Figure 4). As in the kidney, this ring is situated just outside the internal elastica which is preserved for about two-thirds of the circumference and then melts into an area of intensely eosin-stained necrosis in the media. The intima is loose in texture and rather loculated. The cells are large and contain oval, vesicular nuclei. There is an intense periarterial exudate and granulation tissue with many eosinophils. The gland itself shows no change.

Bacteriologic Findings at Necropsy.—Heart-blood: Two broth-flasks show colon bacilli and Gram-positive cocci in chains. The latter were overgrown by the former and were not isolated.

Peritoneal Exudate: Smears show pus-cells and many chain cocci. No other bacteria. Cultures give a pure growth of streptococci.

Pericardial Fluid: Smears show unformed detritus, rarely a pus-cell. No bacteria found. Cultures in broth and ascitic glucose-agar are sterile.

Mesenteric Lymph-Node: Smears show cocci in chains and many pus-cells. Cultures not taken. Unfortunately the cultural characteristics of the streptococcus isolated from this case were not worked out so that it is impossible to compare it with the organism obtained in the second case.

Careful examination of many sections stained for bacteria by various methods failed to show any in the vascular lesions in spite of the fact that the necropsy cultures showed a bacteremia. No spirochetes could be found in sections stained by the Levaditi method.

Case 2.—Girl, aged 10, ill five weeks, was admitted to the hospital Jan. 18, 1914. The family history was negative.

Past History.—Five years ago patient had a slight attack of pain in the epigastrium somewhat similar to the present attack but less severe. It lasted only one day. Had measles as a baby and diphtheria five years ago.

Present History.—Nine days before admission patient was taken suddenly ill in the night with severe pain in the epigastrium. Felt better the next day and was up and about for two days, when the pain came on again and she vomited twice. The pain was sharp and well localized to a small area high up in the midline. Since that time she has been in bed and has had the pain intermittently but always in the same place; has felt feverish, had a headache and has vomited several times. Her throat was sore but has been better for the last few days. She says that she had blood in her sputum at times. No pain is felt on micturition and no blood appears in the urine.

Physical Examination.—Patient well developed and large for her age. Appears acutely ill. No eruption present.

Pharynx: Reddened and shows a considerable thin mucoid, blood-tinged exudate. The left tonsil is enlarged, the right slightly so.

Tongue: Dry and heavily coated.

Lymph-Nodes: The anterior cervical and axillary are palpable.

Heart: Is not enlarged, sounds regular and of good force. No murmurs. Pulses equal and regular. Low tension. Walls not palpable.

Lungs: Clear except for a few transient râles at the left base.

Liver and Spleen: Not palpable.

Extremities: Reflexes normal. No edema.

Abdomen: There is general abdominal fulness and resistance which seems involuntary. No true rigidity. There is tenderness over the whole lower abdomen, more marked on the right side. No dulness in the flanks. No masses. The temperature on admission was 102.6 F. and the blood-count showed 33,100 leukocytes with 93 per cent. of polymorphonuclear leukocytes. A diagnosis of appendicitis was made and she was operated on shortly after entering the hospital. The appendix and peritoneal cavity were found to be normal. The abdomen was explored but no cause found for the symptoms.

January 20: Blood-culture, two broth-flasks sterile.

January 21: There is tenderness and evidence of fluid in both knee-joints and tenderness in both hip-joints. Heart normal.

January 22: There is tenderness in the left shoulder-joint. Temperature between 102 and 103. Wassermann reaction, three plus.

January 23: Shoulder still painful. Signs in the knees have cleared up. Heart normal. Chill.

January 27: Right knee shows a small amount of fluid. Slight amount of edema in the region of the Achilles tendon of both legs. The abdomen is distended in its upper half but not tender. No dulness in the flanks. Spleen not palpable. Heart and lungs normal. There is a little edema of the dependent part of the back.

January 28: Throat culture shows a predominant streptococcus. Patient seen by Dr. Janeway, who believes that she is suffering either from sepsis following a streptococcus sore throat and has a toxic nephritis or that she has nephritis following an unrecognized scarlet fever. Transferred to the medical service. During her stay on the surgical side the urine showed a specific gravity of between 1.012 and 1.028. Albumin, from a very faint trace to a trace. A few to many hyaline and granular casts. No red blood-cells. A blood-count taken January 19 showed 26,200 leukocytes with 92 per cent. of polymorphonuclears, and one taken January 28 showed practically the same. The temperature varied between 101 and 103.6 for a week. It was then lower reaching normal January 27 and on the following two days was between 99 and 100.4. The pulse corresponded with the temperature, varying between 74 and 128. The surgical diagnosis was acute tonsillitis, acute nephritis and acute toxic arthritis. She was apathetic at times, quite thirsty and vomited repeatedly.

On admission to the medical ward her physical examination was unchanged. There was tenderness over the whole abdomen, most marked in the midline above the umbilicus. Very slight edema of the back. The right wrist was somewhat stiff and painful on motion. The right shoulder was somewhat painful.

January 29: White blood-cells, 17,000; polymorphonuclears, 95 per cent.; lymphocytes 5 per cent.

February 1: Liver felt 2 inches below the costal margin. White blood-cells, 52,000; polymorphonuclears, 95 per cent.; lymphocytes, 4 per cent.; large mononuclears, 1 per cent.

February 2: Blood-culture, two broth-flasks sterile. Patient is quite drowsy. There is a mottled erythema over the upper part of her forearms.

February 3: White blood-corpuscles, 66,000; polymorphonuclears, 95 per cent.; lymphocytes, 5 per cent. There is a systolic murmur at the apex. The eruption has disappeared. General condition improved. The right wrist is swollen and painful on motion.

February 5: White blood-corpuscles, 39,800; polymorphonuclears, 90 per cent.; lymphocytes, 9 per cent.; large mononuclears, 1 per cent. Wassermann reaction negative. Blood-culture, two ascitic glucose-agar plates, sterile.

February 6: Metacarpophalangeal joints of both hands swollen and tender. Right wrist better. There is a dusky mottling of the face, whole trunk and extremities, especially over the back and shoulders. It is in the nature of a diffuse erythema.

February 7: Vomitus, 175 c.c. of thick yellow fluid. Total acidity 24, no free hydrochloric acid. Faint reaction for lactic acid. Blood-count: White blood-corpuscles, 40,400; polymorphonuclears, 73 per cent.; lymphocytes, 27 per cent.

February 10: Temperature is lower and joints have cleared up. For the past three days has suffered from paroxysms of abdominal pain and complains of pain on the slightest pressure. There is no rigidity. Rectal examination negative.

February 11: White blood-corpuscles, 26,600; polymorphonuclears, 88 per cent.; lymphocytes, 12 per cent. Vomitus, 155 c.c. of a dark brownish fluid. Faint reaction for blood. Total acidity 8, free hydrochloric acid 4. No lactic acid. Stool: Yellow, fluid; contains small flecks of mucus; moderate reaction for blood; urobilin, moderate. Microscopic examination: Mucus, red cells, pus-cells and epithelial cells. No ova or parasites.

February 14: Patient died. For the preceding week was very apathetic and disliked to be moved or examined. Lay on her side with legs drawn up. At times severe abdominal pains were present which generally required morphin for relief. The pain seemed to be more in the region of the umbilicus. There was tenderness or hyperesthesia over the whole abdomen, but no resistance or rigidity. No signs of fluid. Vomited two or three times a day, generally after taking food. On the day of death, suddenly awoke early in the afternoon, cried out on account of severe abdominal pain, attempted to throw off the bedclothes, became very cyanotic and had a very rapid, almost imperceptible pulse. Relieved by morphin and went to sleep. About three hours later she cried out again, gave a few gasping respirations and died.

Her temperature varied between 99 and 103.2, during the last week between 99 and 101.5. Pulse corresponded, in rate, ranging between 74 and 120. Blood-pressure, from 104 to 60. Urine: Twelve examinations showed the specific gravity quite constant between 1.012 and 1.018, with from a trace to a very faint trace of albumin, generally a very faint trace. Most of the examinations showed hyaline and granular casts. The amount of urine varied between 150 and 1,600 c.c., being 1.000 c.c. or over on only three occasions.

Post-Mortem Examination.—Two and one-half hours post mortem. The body is that of a strikingly pale, moderately wasted young girl. There is a little puffiness of the eyelids and slight edema of the ankles. No subcutaneous nodules found. The abdomen is moderately distended. None of the joints appears swollen. The abdomen contains about 300 c.c. of clear amber fluid. The peritoneal surfaces are smooth and glistening except for a few small shreds of fibrin over the intestinal coils. The appendix is missing, the omentum being adherent to the stump. No nodules seen on any of the vessels. On opening the thorax, the lungs are found partially collapsed and retracted, exposing a large precordial surface. There is about 100 c.c. of clear fluid in each pleural cavity and the same amount in the pericardial sac. The diagnosis was immediately apparent on exposing the heart, the coronary branches showing numerous characteristic nodules (Fig. 5). The heart weighs 320 gm.

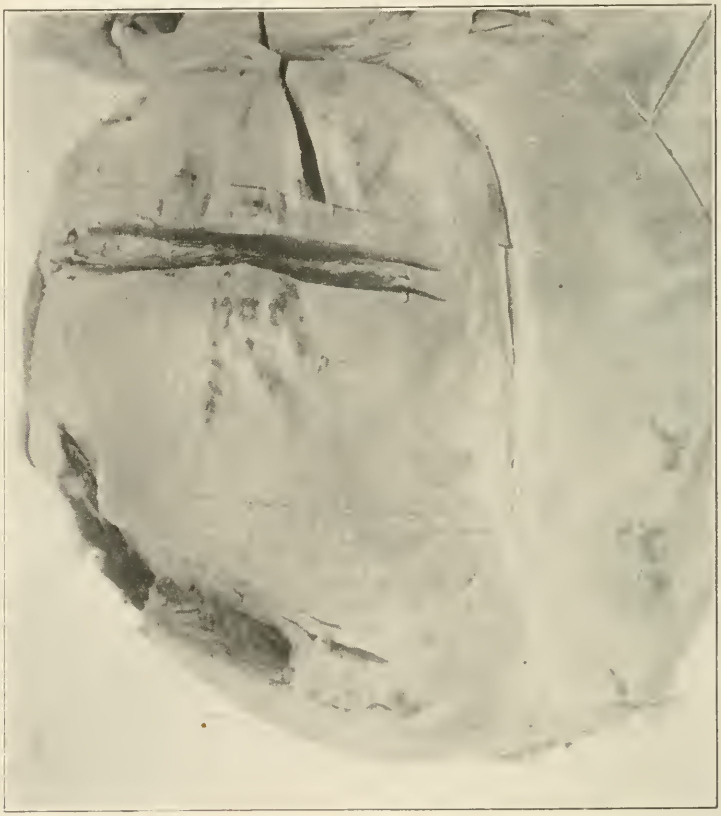

Fig. 5.—Case 2. Heart showing thickly set nodules on coronary branches with overlying petechial hemorrhages.

Right Coronary: At the origin of the infundibular branch there is a globular swelling measuring 6 mm. in diameter, filled with a grayish-red adherent thrombus. Near the termination of this branch there is a small yellowish-white nodule of about 2 mm. in diameter. The transverse branch is almost occluded by connective tissue but shows no nodules. The marginal branch shows a small aneurysmal dilatation, partially filled with thrombus just after it leaves the main artery. In the rest of its course and in its branches are numerous, often closely set, oval and globular, firm swellings, the largest measuring about 3 mm. in diameter. On section, these appear as partially occluded vessels with a very thick whitish periphery. The descending branch shows no external change.

Left Coronary: The main stem is free. The first portion of the descending branch is quite tortuous and contains a thrombus. A little further on there is a bluish-red aneurysm measuring 1 by 0.5 cm. and showing a few petechial spots over it. It is filled with a thrombus. Near the apex there are a few firm nodular swellings. The transverse branch shows similar nodules and in the branches which it gives off over the anterior and posterior surfaces of the ventricles there are numerous firm nodules, the largest measuring 2 or 3 mm. in diameter. Over these, discrete and conglomerate petechial hemorrhages are numerous. A section through any portion of the heart-wall shows the arteries very much

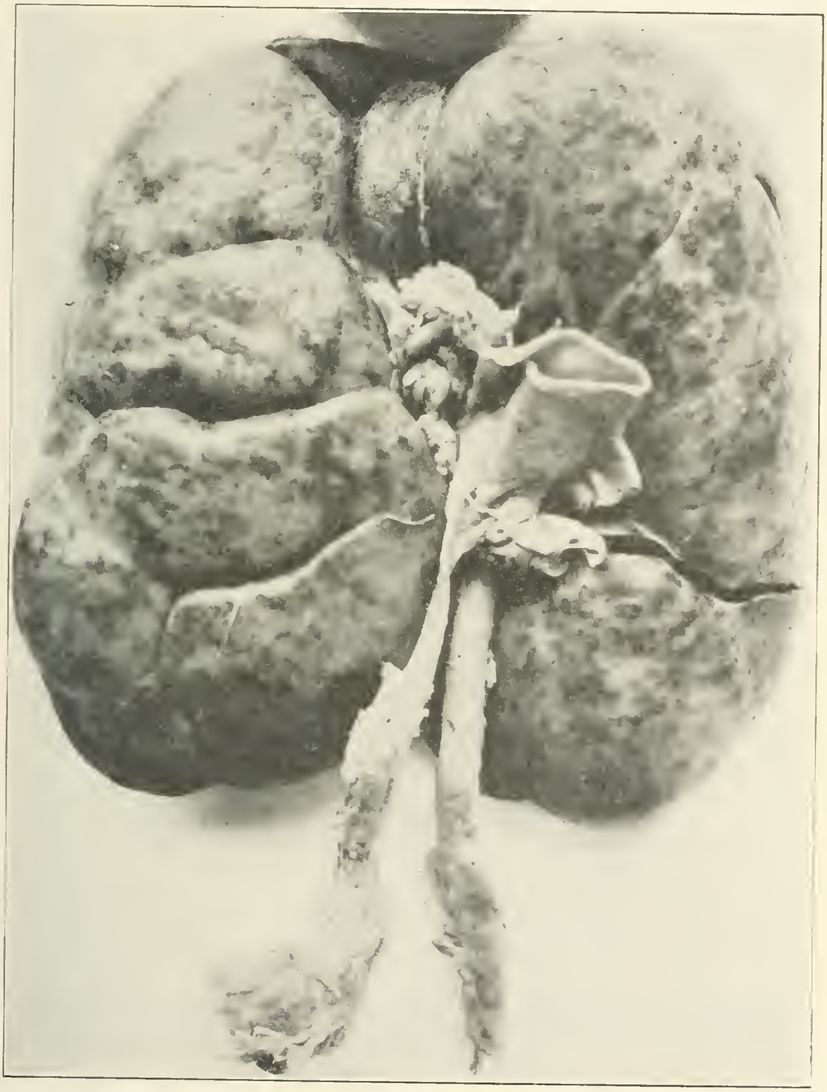

Fig. 6.—Case 2. External view of kidney showing numerous dark raised areas due to hemorrhagic infarcts separated by sunken grayish tissue. The main renal artery and branches show no nodules.

thickened, the lumen generally completely occluded or represented by a minute reddish dot and the adjacent muscle showing irregular yellowish opaque areas. The veins are not changed. The valves and the various chambers of the heart are all quite normal. The aorta and large branches show no change.

Lungs: Except for a moderate collapse, the lungs appear quite normal and one cannot make out any changes in the vessels or bronchi.

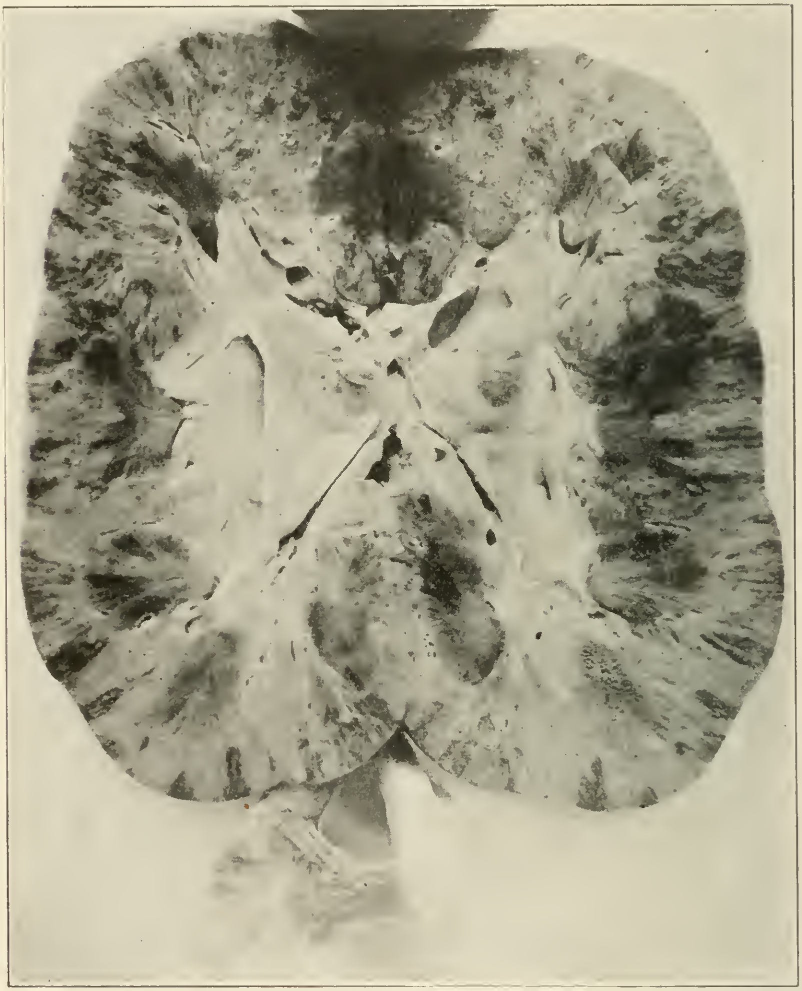

Fig. 7.—Case 2. Same kidney as Fig. 6, seen on section. Shows the infarcts distinctly. The numerous little nodules along the small arteries can also be made out.

Liver: Weighs 1,400 gm. The surface is smooth and brownish yellow with irregular areas of congestion. On section, the tissue about the portal vessels appears a little increased in amount and the vessels a little thickened. No definite nodules seen.

Gall-Bladder: Contains viscid greenish bile. The mucosa appears normal and the ducts are patent.

Spleen: Weighs 160 gm. The capsule is tense but smooth and the organ is quite firm. The cut surface remains quite flat and the pulp is firm. The color is purplish red and the malpighian corpuscles are unusually numerous and larger than normal. One obtains the impression that the small central arteries are slightly thickened.

Pancreas: Is large and of normal consistency. On section, it shows nothing abnormal. No macroscopic lesions of the arteries can be seen.

Stomach: Appears quite normal. No lesion of any of the vessels.

The esophagus, trachea, larynx, thyroid and parathyroids appear normal.

Kidneys: Are quite alike (Figs. 6 and 7). The left weighs 160 gm. and the right 150 gm. The fatty capsule is moderately thick but strips easily. The true capsule is easily removed without tearing the renal surface which has a most remarkable appearance. It is coarsely irregular and in color, a mottled red and opaque yellowish gray with a sprinkling of small yellowish points. The raised portions are a deep red with small gray dots. They are abundant, in the aggregate representing fully one-half of the surface, are quite irregular in outline, several often running together to form map-like patterns and they are separated by slightly sunken, dull, opaque, yellowish-gray tissue containing flecks of yellow. The same appearance is seen on section. The cortex averages from 6 to 8 mm. in thickness. The vessels of the pyramids are much congested. The cortex and tissue between the pyramids has a rather rough, furred appearance and one can make out numerous, small, hard pearly gray nodules along the course of the vessels. These resemble miliary tubercles. There are many, larger and smaller, deep red and more grayish-red, necrotic-looking, roughly wedge-shaped areas extending from the surface deeply into the cortex and in places into the medulla. These correspond with the raised areas on the surfaces. They are separated by slightly sunken, yellowish-gray opaque tissue in which are tiny nodules and flecks of yellow. The main renal arteries and their larger branches show no change. An attempt was made to inject the kidney but no gelatin could be forced through the vessels. The kidneys were almost exactly like the colored illustration in Dickson's article.

Adrenals: These are of normal size. On section there are several small vessels which seem thickened and show thrombi.

Uterus: Undeveloped. The tubes, vagina and ovaries all appear normal.

Intestines: Duodenum normal. In the ileum, there are two small, widely separated areas which show a little hemorrhage of the crests of the rugae. In the cecum, there is a small cavity at the appendix stump filled with reddish, grumous material. Otherwise the intestines show no change and the vessels are quite normal in appearance.

Omentum and mesentery are normal.

Mesenteric lymph-nodes: Moderately enlarged and show some thickened vessels with thrombi but no definite nodules.

Arteries: The celiac axis and its branches show no change. In fact, none of the larger arteries shows anything abnormal. The veins are quite normal. The blood in all the vessels is quite fluid. Permission could not be obtained to examine the nervous system, joints or extremities.

Microscopic Examination.—Heart: In one section, taken at a distance from the region of the nodules, the muscle appears fairly normal. The striations are distinct and the vessels quite normal but congested. Around one small vessel there is a little infiltration with wandering cells penetrating between the muscle fibers. Among these cells there are a few eosinophils. In another section there are several irregular areas of fibroblasts replacing the muscle-fibers. A section through the largest aneurysmal nodule shows a fair sized, dilated vessel completely filled with an adherent thrombus which shows an invasion of fibroblasts from the vessel-wall. The wall is much changed. Only in one small area is the internal elastica present and here it is in direct relation with the thrombus. Immediately external to it there is a ring of intensely eosin-stained material in which nuclei are still seen. In places, the media appears rarefied and shows spaces, in other regions the vessel-wall is composed of fibrous granulation tissue. The adjacent heart-muscle shows granular degeneration and irregular fibrous-tissue replacement. Near the aneurysm there is a smaller vessel seen in cross-section. One can make out a delicate endothelial lining, external to which there is a very loose tissue, internal elastica, homogeneous hyaline ring and a marked adventitial infiltration with mononuclear wandering cells and fibroblasts, among them an occasional eosinophil. The adjacent heart-muscle is granular and degenerated and shows considerable fibrous tissue. Another vessel is seen in which the infiltration is largely perivascular. A final heart-section shows a large artery filled with thrombus showing beginning organization about the periphery. The heart-muscle is cloudy and shows scattered wandering cells and considerable fibrous tissue. In the artery, the elastica is quite gone. The homogeneous hyaline ring is quite distinct close to the intima and at one point it splits into two layers enclosing a granulation tissue containing beautiful fibroblasts and hemorrhage. The perivascular tissue is very cellular, showing fibroblasts, wandering cells and many eosinophils. A longitudinal section of a small vessel shows the coats normal but a considerable similar cellular exudate in the perivascular tissue. Eosinophils are numerous. A cross-section of another vessel shows the lumen filled with beautiful granulation tissue and in another there is canalization of the thrombus.

Aorta: Appears normal. Several small branches at the side of the aorta show characteristic changes of a rather late type.

Lungs: The lung-tissue shows slight collapse with some thickening of the alveolar walls. Sections taken near the surface show no vascular lesion either in the pulmonary or bronchial arteries. Sections from the hilum show very pretty lesions of the bronchial arteries but none of the pulmonics. There is a small lymph-gland present in which eosinophils are very numerous.

Bronchial Glands: The follicles are moderately well shown. The sinuses are dilated and contain relatively few cells. One or two small arteries are seen which show the changes seen elsewhere, eosinophils being quite numerous. Some of the follicles show hyaline degeneration.

Spleen: This is much congested, the blood-spaces showing up very clearly. In some of the malpighian corpuscles there is hyaline degeneration of the central cells. In one section, there is an irregular area of infarction. Eosinophils are quite numerous throughout. Several of the small arteries show characteristic changes. In some, the lesion appears as an adventitial cellular infiltration, with a rather marked invasion of the media with wandering cells and fibroblasts, the muscle being almost entirely replaced. In one or two of the small arteries of the malpighian corpuscles there is a fringe of cells just outside the adventitia, most of which are eosinophils.

Liver: The cells show cloudy swelling and in many places vacuoles. In most of the portal spaces there is an infiltration of wandering cells and eosinophils, often quite definitely about the arteries. In a few of the arteries the lesion is quite definite. Another section so taken as to give a longitudinal view of the vessels and bile-ducts gives a very good picture of the lesion and shows considerable surrounding fibrosis.

Pancreas: The lesions here are exquisite. In general, the parenchymal cells and islands are normal, but there is a moderate increase in the fibrous tissue between the lobules. In one region, there is a definite area of necrosis, apparently an infarct, the supplying arteries being severely changed. The striking thing in the section is the lesion of the vessels. Almost every artery shows most extensive changes and there is a very marked perivascular wandering-cell infiltration with fibrosis. One of the prettiest lesions in the whole case is seen in a longitudinal section of a small artery (Fig. 8). At one end the artery is perfectly normal. It then shows a wider and more irregular lumen with a moderately thickened intima in which are various-sized irregular nuclei, an internal elastica which is more or less preserved until one reaches the region of most extensive change where it gradually fades out, a homogeneous hyaline band on either side of the vessel which is in places internal to the elastica, in other places external and for some distance divided by it. External to this one cannot differentiate between media and adventitia. One comes on a loose mass of various-sized wandering cells and eosinophils with a few fibroblasts and outside of these a young connective tissue with numerous eosinophils. In serial sections, the lesion can be traced into normal artery once more.

Esophagus: The lesion is seen in one or two of the small arteries of the serosa.

Stomach: Normal. One small artery is seen in the muscularis with a little cellular infiltration about it.

Colon: A longitudinal section of a small vessel shows it normal but on either side of its wall the branches show extensive changes.

Ileum: Normal.

Cecum: One vessel shows the lesion. External to the cecum there is an area of granulation tissue the borders of which are necrotic. This represents the softened area at the base of the appendix described in the gross. Appendix is normal.

Mesenteric Lymph-Nodes: Appear much the same as the bronchial.

Lymph-Node at Gall-Bladder: Shows various areas of hyaline degeneration but no vascular lesions.

Thyroid: Normal. Vessels unaffected.

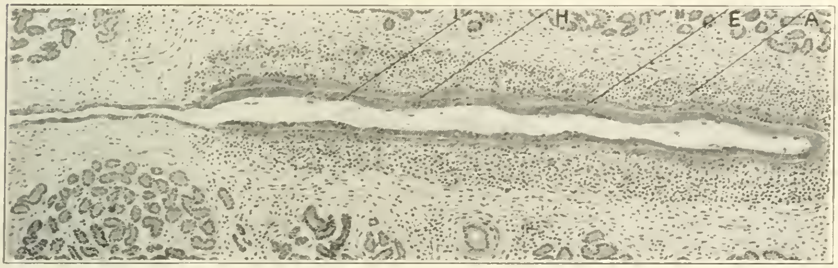

Fig. 8.—Case 2. Longitudinal section of artery in pancreas. The rather abrupt beginning of the lesion is well seen at the left. The intima, *I,* is thickened and is indistinguishable from the hyaline ring, *H.* The internal elastica, *E,* thus apparently divides this hyaline ring. The media is represented by the hyaline ring and the adventitia, *A,* merges with the periarterial infiltration. In other sections the lesion can be traced to the right into normal artery again.

Kidneys: Sections show very numerous fresh hemorrhagic and older infarcts. A large portion of the cortex is thus affected and the infarcted areas often run down to and into the medulla. The small amount of intervening renal tissue is fairly normal except for the vascular changes which are very extensive, almost every small artery being affected. There is considerable perivascular infiltration. One fairly large artery shows an extensive thickening of the intima, the internal elastica remaining intact. The thickening consists of a bluish-pink material rich in spindle-shaped fibroblasts, many of the cells having peculiar little spaces about them. The media is composed of a similar tissue while the adventitia is more fibrous. The other vascular lesions are merely repetitions of the changes seen elsewhere.

Adrenals: Several of the small arteries about the organ show characteristic lesions. There are several infarcted areas in the cortex.

Fallopian Tube: One vessel found which shows typical changes.

Ovary: Normal.

Psoas Muscle: One small artery is seen which shows a beginning infiltration about the adventitia. The muscle-fibers are normal.

Pectoral Muscle: Very distinct changes are seen about many of the small arteries, the muscle-fibers remaining unaffected.

Uterus and Bladder: Normal.

Bacteriologic Findings at Necropsy.—Heart-Blood: Cultures were taken in various mediums, two ascitic glucose-agar shake-cultures, three ascitic glucose-agar slants and one neutral broth. These all remained perfectly sterile. At the end of three weeks they were examined, although there had been no sign of any growth. At present, three months after the necropsy, no growth has appeared.

Peritoneum: Cultures showed a *Staphylococcus aureus.*

Pericardial Fluid: One tube remained sterile, the other showed a colon bacillus. The most promising field for obtaining successful cultural results seemed to lie in the examination of the cardiac nodules and consequently most of the attention was directed to them. In the first place numerous smears were made from these nodules. These were stained by various methods but gave entirely negative results. The largest aneurysmal nodule was excised and washed in three changes of sterile salt solution. It was then divided into two portions, using Rosenow's sterile method. One portion was ground up in sterile salt solution. Of this, one guinea-pig received 0.5 c.c. intraperitoneally, one received 1 c.c. intraperitoneally and two rabbits received 0.5 and 1 c.c. respectively, intravenously. All of these animals remained perfectly well. They were finally killed, May. 1, two and one-half months after inoculation. None of them showed any lesions and the microscopic sections of the various organs failed to reveal any changes.

The second portion of the nodule was again divided and after flaming the pieces were planted at the bottom of two ascitic glucose-agar tubes. The rest of the bacteriologic work on the case was done by Dr. Coulter, and I am indebted to him for the following facts: After eighteen hours, both of the tubes were clouded. Direct examination and subcultures showed *Bacillus subtilis.* The nodules were at once removed, held within a flame until there was free spattering of fat and the entire surface was shriveled and discolored and then were replanted in ascitic glucose agar. The nodules sank to the bottom of the tube before the medium hardened. The ascitic fluid used in these cultures had been repeatedly tested for sterility. One tube has remained perfectly sterile. One tube showed no growth for two weeks.

On the sixteenth day there were two separate areas of clouding at the bottom of the tube, between the nodule and the glass. Each area was somewhat fan-shaped with the wide portion of the fan directed away from the nodule. Three days later there was very slight if any increase in the areas of clouding. A long sterile pipet was introduced to the bottom of the tube encountering considerable resistance owing to the firmness of the medium. At the very bottom, however, about the nodule, there were found several tenths of a cubic centimeter of slightly turbid fluid. About 0.1 c.c. of this fluid was inoculated into each of three ascitic glucose-agar cultures, one being a surface slant, the other two deep cultures similar to the one from which the fluid was withdrawn. Smears of this fluid showed rather large Gram-positive cocci, mostly in chains of from four to ten, but occasionally arranged as diplococci or tetrads. About the latter there was an indistinct capsule. On the following day the subcultures showed the following:

Surface cultures: Profuse growth, for the most part confluent with very numerous, uniformly distributed, more opaque areas. Growth emulsifies easily. It is not very viscid but strings out slightly when touched with the loop. The medium is not precipitated but the growth has extended between the medium and the glass. The medium was freshly slanted before inoculation so that it was quite moist.

Deep Cultures: Uniformly clouded throughout. No gas bubbles or cracks in the medium. Smears showed organism identical with those in the original culture. Smears from the surface growth showed the cocci smaller, somewhat flattened and generally arranged as diplococci, chain formation being very slight. Plates were made from all of the cultures. In all there was but one type of colony in twenty-four hours. This was large, 1.5 by 2.5 mm. in diameter, moist, surface convex and glistening, outline smooth and regular, color uniformly grayish, consistency only slightly viscid.

Growth in peptone broth shows a uniform clouding with very slight sedimentation in six hours. In twenty-four hours there is marked sedimentation, but the fluid remains uniformly cloudy. Smears show chains of ten or more cocci with a distinct capsule. The organism is not bile-soluble and shows the following fermentation reactions: Dextrose +, dextrin +, saccharose +, lactose +, galactose +, inulin —, raffinose —, mannite —, salicin +, litmus milk +, acid, coagulated but not dissolved.

Animal inoculations were made from the second generation of deep cultures. An emulsion was made up to the strength of 500,000,000 to the cubic centimeter and of this a guinea-pig received 0.75 c.c. intraperitoneally, a rabbit 1.5 c.c. intravenously and a monkey 1.5 c.c. intravenously. None of these animals showed any reaction. They continued perfectly well and were killed two months later. They showed no gross or microscopic lesions. Another rabbit was given 5 c.c. of an emulsion containing 1,000,000,000 to the cubic centimeter. Death resulted in eighteen hours. The organism was recovered at the necropsy. There were no gross or microscopic lesions of the vessels, especially no signs of inflammation about the vessels or in the perivascular lymphatics.

Pieces of tissue were preserved in alcohol, formaldehyd and Zenker's fluid and sections were stained by various bacterial stains. A careful search was made of many sections for bacteria, without results. In some of the sections, especially those of the pancreas, there were some small bodies resembling cocci, but they could not be differentiated from the numerous particles of chromatin present. A set of sections was also stained by the Levaditi method but with entirely negative results.

The foregoing results have been reported in detail because they represent the most extensive bacteriologic investigations yet made on the disease. The examinations can be considered quite negative with the possible exception of the streptococcus isolated from the cardiac nodule. The occurrence of a *Staphylococcus aureus* in the peritoneal fluid seems to be merely a coincidence. Had it any real relation to the disease, it seems inconceivable that it should not have been obtained from the other cultures both during life and at the necropsy. As the cardiac nodule showed a contamination at the start it is, of course, quite possible that the streptococcus was one likewise. Against this is the fact that the nodule was very thoroughly seared and that the growth did not appear until a considerable period of time had elapsed. Even if this organism did come from the nodule it may have been there merely as an accidental secondary infection. As the occurrence of a streptococcus has been the only moderately constant finding in necropsy cultures of other cases it has seemed important to me to include the cultural characteristics of the one found in this case for the purpose of comparison in case any similar organism is isolated in the future.

The animal inoculations have proved nothing except that the disease was not transmitted to them either by the direct inoculation of material from the nodules or by the streptococus isolated. It is quite possible that the disease cannot be transmitted to these animals. It would have been interesting to have inoculated stags with material from the nodules, but unfortunately this was not possible.

CASES REPORTED UP TO THE PRESENT TIME

The following is a list of cases of periarteritis nodosa reported up to the present time:

Rokitansky, 1852. Studied microscopically by Eppinger thirty-five years later.
Kussmaul and Maier, 1866.
Zimmerman, 1874.
Chvostek and Weichselbaum, 1877.
P. Meyer, 1878.
Eppinger, 1887. Kundrat's case and microscopic description of Rokitansky's.
Fletcher, 1892. Necropsy by von Kahlden.
von Kahlden, 1894.
Graf, 1896.
Rosenblath, 1897.
Freund, 1899.
Krzyszkowski, 1899.
P. Müller, 1899. Two cases.
Schmorl, 1903.
Ferrari, 1903.
Veszprémi and Jancsó, 1903.
Schreiber, 1904.
Mönckeberg, 1905.
Versé, 1905.
Benedict, 1907.
Oberndorfer, 1907.
Bomhard, 1908. Same case as that reported by Oberndorfer.
Benda, 1908. Two cases.
Dickson, 1908.
J. E. Schmidt, 1908.
Longcope, 1908.
Hart, 1908.
Künne, 1909. Same case as that reported by Hart.
Datnowski, 1909.
Beitzke, 1910. Same case as that reported by Datnowski.
Moriani, 1910.
Cooke, 1911.
Veszprémi, 1912. Two cases.
Lewis, 1912.
Jonas, 1912.
Beattie and Douglas, 1912-13.

REVIEW OF REPORTED CASES

So far as it is possible to decide, the preceding thirty-eight cases are true cases of periarteritis nodosa. Up to the present time it has been impossible to be sure of the diagnosis without a microscopic examination of the lesion. Consequently, a case such as that reported by Sabin cannot be included in this series, although it may well have been an instance of the affection. In order to make the record complete, several of these questionable cases are given at the end of this article. These have either been reported as periarteritis nodosa or have been considered such by some authors.

The following review is based on a study of the preceding thirty-eight cases. It is at once apparent on reading over the reported cases that many are lacking in important details. The more detailed report of additional cases will be necessary to bring the clinical picture to completeness. There is sufficient material, however, to bring out many facts of interest and to give a clinical picture which will at least aid in the recognition of future cases.

Historical.—In 1852, Rokitansky, in a paper entitled "The Formation of Aneurysms of the Arteries in General, Except the Aorta, and Most of Its Important Primary Branches, with the Further Exception of the Cerebral Arteries,"[2] gave the first complete gross description of the disease. Thirty-five years later, Eppinger studied and described the histologic changes in the arteries of the mesentery in this case. To Kussmaul and Maier, however, must go the credit for the first complete gross and microscopic description of periarteritis nodosa and its recognition as a distinct disease. They reported a case in 1866 as "A hitherto undescribed peculiar disease of the arteries which is accompanied by Bright's disease and a rapidly progressive general paralysis of the muscles,"[3] and proposed the name by which the disease has since been known.

There is some ground for believing that the disease was grossly recognized as early as 1755 by Michaelis and Matani, and in 1810 Pelletan reported very briefly a case in which he counted sixty-three small aneurysms on the various arteries. These reports are too brief, however, to admit of any conclusions as to the true nature of the process. From the time of Kussmaul and Maier's publication to 1899, but eight additional cases were reported, or an average of about one case in four years. Then, as the disease became better known, the number of cases showed a considerable increase, twenty-eight being reported in the following sixteen years. Five of the cases are from the United States and two from Great Britain, nearly all of the rest being from Germany.

Name of the Disease.—As one might expect, several names have been proposed to supplant Kussmaul and Maier's "periarteritis nodosa." Thus we have "multiple aneurysmen" of Meyer, "das congenitale aneurysma" of Eppinger, "arteritis nodosa proliferans" of Fletcher, "polyarteritis acuta nodosa" of Ferrari, "meso-periarteritis" of Hart and "panarteritis," "arteritis nodosa" or "polyarteritis nodosa" of Beitzke, according to the view of the author as to the nature of the process. While it is possible that some of these names are a little more accurate than the one originally used, the disease has come to be known under the title "periarteritis nodosa" and it seems better to continue this name, at least until the etiology of the disease has been established, rather than to introduce confusion by using one or other of the various substitute titles proposed.

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[2] Aneurysmenbildung sämmtlicher Arterien mit Ausnahme der Aorta, und der meisten ansehnlichen primitiven Aeste derselben, ferner mit Ausnahme der Gehirnarterien.

[3] Eine bisher nicht beschriebene eigenthümliche Arterienerkrankung die mit Morbus Brightii und rapid fortschreitender allgemeiner Muskellähmung einhergtht.

Etiology.—The fact that the etiology of periarteritis nodosa is still quite unknown lends added fascination to the study of the disease and emphasizes the desirability of recognition of cases during life in order that complete bacteriologic studies may be carried out. The history of the etiologic views falls into three main groups.

Syphilitic: Influenced by Virchow's opinion, Kussmaul and Maier were inclined to the view that the disease was a syphilitic affection of the arteries. Chvostek and Weichselbaum, Graf, Schmorl and especially Versé have been the chief exponents of this view, while Müller and Benedict have found it impossible to rule out. Many pages have been written upholding and combating syphilis as the causative factor. It would lead too far afield to go into all the arguments. Suffice it to say that the great majority of observers are opposed to the idea even when they limit themselves to purely histologic studies; the Wassermann reaction has been negative in the few cases in which it has been applied; no one has been able to find spirochetes in the lesions; a syphilitic history fails in most of the cases; in only a very few have any other syphilitic lesions been found at necropsy, and a similar, if not identical, disease occurs in stags as pointed out by Lüpke.

Mechanical: While no one seriously considers this view at the present time it is interesting historically. Meyer, in 1878, believed that the disease was due to high blood-pressure rupturing the media, which was either imperfectly formed or later injured by some toxic substance such as alcohol. Nine years later, Eppinger upheld the view of a congenital debility of the internal elastica with rupture. A variation of the mechanical theory is encountered in Ferrari's idea that the lesion is produced by the central or local action of a toxic substance, such as alcohol, causing a paralysis of the smooth muscle-fibers. All of the facts are against these various ideas and it is scarcely worth while to discuss them.

Infectious or Toxic: All observers, with the exception of those above mentioned, believe that periarteritis nodosa is the result of an acute infection, the arterial lesion being caused by bacteria or their toxins. The nature of the infecting agent is still unknown. Cultural investigations during life have been few and yet it is rather striking that in the four cases in which blood-cultures have been taken the results have been quite negative. In Datnowski's case these were repeated three times and the same was true in our second case, the last blood taken being plated in ascitic glucose-agar. While in no way conclusive, these results would seem to point against any one of the commoner organisms as the causal factor. Anaerobic cultures have not been taken. In our second case, ascitic glucose-agar shake-cultures were made from the heart's blood post mortem, but also with negative results.

Another striking fact is that the examination of sections of the lesions for bacteria has almost uniformly failed to show any organisms. Graf found a few short rods in his sections, but felt that they could not be considered, as the necropsy was done many hours post mortem. Longcope found a few chain cocci in sections from the kidney, but they were not in relation to the nodules.

In only eight cases have cultures been taken at necropsy. In one instance they were accidentally destroyed, leaving the following seven observations:

Oberndorfer: *Staphylococcus aureus* cultivated from the kidney and spleen.

Datnowski: Streptococcus cultivated from the heart's blood. There was a terminal perforative peritonitis in this case.

Veszprémi: Cultures sterile.

Jonas: Streptococcus obtained from the kidney and an influenza bacillus from the adrenal.

Beattie and Douglas: Streptococcus obtained from the renal hemorrhage.

Lamb, Observation I: Streptococcus from the heart's blood associated with a colon bacillus. Streptococcus from the peritoneal exudate. In this case there was a vegetative endocarditis and terminal peritonitis.

Lamb, Observation II: Streptococcus from the cardiac nodule. *Staphylococcus aureus* from the peritoneum and colon bacillus from the pericardial fluid.

Thus, in all but one case in which cultures have been taken, some organism has been obtained. In five of these it was some variety of streptococcus. In two out of these five there was some complicating condition and in two there was also some other organism isolated. In Longcope's case a few chain cocci were found in the kidney sections, but not in association with the nodules.

In reviewing the etiology, one finds no support for the syphilitic or mechanical theory. The course of the disease, the irregular temperature, the high leukocytosis, the complications and the nature of the lesion all point to some infection. Cultures taken during life have been uniformly negative, but have also been limited in number. The failure to demonstrate any bacteria in the lesions may indicate their early disappearance as is supposed to be the case in rheumatic lesions. While one is not justified in excluding the occasional finding of such an organism as the *Staphylococcus aureus* or influenza bacillus in postmortem cultures, the most striking point in the examinations so far made is the occurrence of some variety of streptococcus in five of the seven cases, or in 71 per cent. In another case they were seen in sections. It is for this reason that we have included a full description of the streptococcus isolated in our second case. Animal inoculations with this organism and with material from the nodules have been negative.

At present, one can do no more than call attention to these facts as they have occurred. The final solution rests with the future.

It is contended by some that the disease is not specific, but that it is merely an acute infectious lesion of the arteries due to any one of a number of bacteria.

Heredity.—There is no indication that it plays any part.

Sex.—Of the reported cases, twenty-eight were in males, nine in females and in one the sex is not stated. Thus it would appear that the disease is about three times more frequent in males than in females.

Age.—It is essentially a disease of adolescence and early adult life — 71 per cent. of the cases occurring between the ages of 10 and 40. It is not limited to this period, however, the youngest case being in a child 2½ months old, and the oldest occurring at the age of 57. The number of cases in the second decade is slightly larger than in any other. The following table shows the number of cases in each decade:

Years	No. of Cases
1-10	1
10-20	10
20-30	8
30-40	9
40-50	5
50-60	5

Occupation.—This is most varied and it is quite impossible to discover any connection with the affection.

Past History.—In about one-third of the cases this is not given. In only four cases is there a definite history of syphilis. In a few there is a history of symptoms similar to but antedating the symptoms of onset by some months or years. In a few there is a history of some immediately preceding infection. There is thus no apparent relation to any other disease, and one gathers the impression that the previous illnesses are entirely irrelevant. It is, however, interesting to note that some cases do show similar symptoms in their past history either as isolated occurrences or in recurring attacks.

Symptoms.—Onset: In the 32 cases in which this is mentioned, we find a more or less acute invasion in 14 cases and a more gradual onset in 18. The symptoms of onset in order of frequency were as follows: Pains in the muscles or joints, 13; fever, 9; abdominal pain, generally in the epigastrium, 8; edema, 8; weakness, 5; disturbances of sensation, 4; diarrhea, sweating, purpuric eruption, sore throat, 3 each; chilliness, headache, vomiting, anorexia, constipation, cough, 2 each; and scattered symptoms of dizziness, urticaria, pain in the chest, eruptions, hemoptysis, cyanosis, dyspnea, icterus, swelling of the testis and asthma.

General: Mentioned in 32 cases. Weakness, generally progressive, 24; emaciation, 13; sweating, 7; jaundice, 6; sleeplessness, 4; chilliness, 4.

Subcutaneous Nodules: In two cases (Schmorl, Benedict) the diagnosis has been made during life by the examination of excised subcutaneous nodules. Everyone has been impressed with the importance of these nodules and it is generally agreed that they offer the only certain means of diagnosis. And yet they were present only eight times or in about 21 per cent. of the cases. In Kussmaul and Maier's case, small, firm, pea-sized nodules appeared beneath the skin of the abdomen and thorax four days before death. Freund merely states that there were subcutaneous nodules of the face, neck and arms noticed during the patient's eight days in the hospital. Müller (Case 1) found the nodules about one knee at the necropsy.

Schmorl reports very briefly the case of a woman who showed nodules in the abdomen and thigh which on excision were characteristic of the disease. They were apparently cured by antisyphilitic treatment. Mönckeberg noticed a few, small, elastic, painless nodules in the thigh on the day before death. Benedict has described the nodules in more detail. At the end of the fourth week, in his case, a fist-sized tumor appeared in the abdomen and increased somewhat in size. This was finally considered an intramuscular hematoma. At about the same time, four or five hazel-nut-sized, round, painless, easily movable nodules appeared in the skin of the thorax and several pea-sized nodules in one leg and in both forearms. A thoracic nodule showed only clotted blood. Later, eleven pea-sized, round or oval, moderately hard, freely movable, nodules were found under the chin and in the buttocks, arms and legs, three hazel-nut-sized tumors under the left breast and a goose-egg-sized nodule containing pus under the left trochanter. A second small nodule removed from the leg showed changes characteristic of the disease. In Datnowski's case there was slight periosteal thickening of the tibia, and four days before death small elevations of the abdomen appeared. It was suggested that both these represented subcutaneous nodules, but no examination was made. In the first of the cases here reported, there was a peculiar small firm area in the course of the internal saphenous vein, a raised reddish-brown area 3 by 1 cm., in the right thigh, lying over a superficial vein and the early appearing goose-egg-sized hematoma-like swelling of the ankle. These were not in evidence at the necropsy and so, unfortunately, no examination was made.

Superficial Lymph-Nodes: These were noted as enlarged in only four cases.

Edema: The most striking fact in this connection is that in twenty-one, or 61 per cent., of the thirty-four cases in which histories are given, there was edema at some time during the course of the disease. In only two cases is it definitely stated to have been absent. In the eleven remaining cases in which the histories are more or less completely given it is not mentioned and was presumably absent. The edema was generally of the ankles, less often of the face or body.

Skin Eruptions: In seven cases there was an eruption of hemorrhagic character, petechial, purpuric or ecchymotic and at times this was associated with urticaria. In a few instances, such hemorrhagic spots were the first things noticed at the onset of the disease, at other times they appeared during the course, generally in recurring crops. In one case there was an eruption of uncertain character, considered to be that of either syphilis or scarlet fever. In another there was a desquamation of the whole body typical of scarlet fever, but without any known preceding eruption. In one of our cases there was a rather late diffuse erythema of the face and body.

Pains in the Joints: In fifteen cases, or 39 per cent., there were definite joint-pains, often quite severe, usually involving more than one joint and behaving very much like acute rheumatic fever.

Pains in the Muscles: In eighteen cases, or 47 per cent., there were pains in the muscles, generally of the extremities, usually quite severe and described as "tearing." In three cases these pains were associated with distinct muscular atrophy.

Temperature: In four cases no mention is made of the temperature. In two cases it is definitely stated to have been normal, while in the remaining thirty-two there was fever. Thus 94 per cent. of the cases have shown a definitely febrile course. In some, it is merely stated that there was slight fever, in others it is perhaps a little difficult to separate the fever of the disease from that of the complications. In reviewing the cases in which there is more detailed information, it is apparent that the temperature is generally irregular, at times reaching 104 or even 105 F., and at other times being subnormal. It is apt to be of a remittent type, the remissions being accompanied by marked sweating.

There are often periods of several days when the temperature is quite normal.

Circulatory System: Symptoms and physical signs directly referable to the heart and vessels are rather strikingly absent. Thus in our second case in which the coronary arteries were most extensively involved, there were no signs to direct one's attention to the heart until near the end. In 7 cases there was enlargement of the heart, murmurs in 7, dyspnea in 5, cyanosis in 4, precordial pain in 1 and pericarditis in 1.

Blood-Pressure: There is information on this point in only five cases. In Benedict's case it was 140, in Datnowski's 215, in Künne's 77 to 100, in the first of our cases 130 to 165, and in the second 104.

Pulse: In five cases the pulse was not mentioned, and in one it was quite slow. In the remaining thirty-two, or 97 per cent., it was increased in frequency, and in sixteen, or in one-half of the cases in which it was increased, it was more rapid than one would expect from the height of the temperature.

Blood: Anemia is a striking feature of the disease. It is stated definitely as being present in 55 per cent. of the cases and is generally described as being most conspicuous. In only five cases is there any record of a red cell-count or hemoglobin estimation. In these counts the red cells varied from 3,500,000 to 2,800,000, and the hemoglobin from 75 per cent. to 42 per cent. Leukocytosis: Observations have been made on the white cells in only twelve cases. Leukocytosis failed in only one case, that of Dickson, in which there was an associated tuberculosis of the lymph-glands and lungs. In two cases it is simply stated that there was a leukocytosis. In the nine cases in which the figures are given, the count varied from 9,000 to 66,000. The low count was in a case in which other, later counts were as high as 24,000. The count is generally high, between 16,000 and 30,000. In only four cases were differential counts made. Of special interest in this connection is the fact, first mentioned by Datnowski, that the eosinophils are increased. In his case they were 10 per cent. In Lewis' case they were from 22 to 30 per cent., and in our first case from 32 to 58 per cent. In all of these cases the usual causes for eosinophilia were excluded. In our second case they were not increased. It is most important to have more observations on this most interesting point, as it may furnish a most valuable means of diagnosis. It is rather significant that the cellular infiltration of the arterial lesions is generally rich in eosinophils. The polymorphonuclear leukocytes are increased in number. Blood-Cultures: Taken in only four cases and sterile in all. In Datnowski's case they were repeated three times. In our second case, three different cultures were taken and one of these was plated in ascitic glucose-agar. Wassermann Reaction: Taken in only three cases and negative in two. In our second case one test was positive and one was negative. The first was taken when the temperature was high. We have become very suspicious of the accuracy of positive reactions in the presence of high fever. The second test we believe more reliable, as the temperature was low. In one case a negative reaction was obtained with cadaver blood.

Respiratory System: The respiratory symptoms bear very little relation to the disease with the possible exception of the asthma, which is at times cardiac, and the blood in the sputum. In one of our cases asthma was a most striking feature from the outset, and in one other of the reported cases it was severe. In three cases there has been blood in the sputum. The other symptoms have been the following: cough in 8; pulmonary edema, pneumonia, pain in the chest, 2 each; pleural effusion, emphysema and hemoptysis, 1 each.

Gastro-Intestinal System: A large number of the cases show some symptoms referable to the gastro-intestinal system. In order of frequency they have been the following: Vomiting, 17; anorexia, 9; constipation, 7; diarrhea, alternating with constipation, 5; diarrhea, 4; blood in the stools, 1; *Ascaris lumbricoides,* 1. In the only 3 cases in which gastric analyses were made the free hydrochloric acid was either reduced or entirely absent.

Abdomen: The abdominal symptoms are most important. It is this aspect of the disease which is of interest to the surgeons, for at times the symptoms have been severe enough to persuade one that an acute surgical condition existed. The second case here reported is a striking illustration of this. In seventeen cases, or 50 per cent. of those in which the facts are given, there was abdominal pain. In one-half of these it was located in the epigastrium or upper part of the abdomen. In the rest it was generally diffuse over the whole abdomen. In three cases it was in the region of the kidney. In eight cases the abdominal pain was one of the marked symptoms of onset. The character of the pain is fairly constant. In most cases it comes on in recurring attacks and is severe, colicky or cramp-like. At times it is severe and constant for several days at a time. In a few cases it radiates to the back. It is generally associated with abdominal tenderness and distention. In seven cases the liver was palpable and in four cases the spleen could be felt.

Urogenital System: The lesion affects the small arteries of the kidney in nearly every case. Therefore, the symptoms referable to the kidney are most important. In ten cases no mention is made of the urine. In five cases it is definitely stated that albumin was not present. Often, however, there was only one examination of the urine and the condition of the kidneys at necropsy suggested that, in some of the cases at least, albumin would have been found had repeated examinations been made. In twenty-three cases, or eighty-two per cent. of those in which the condition of the urine is given, albumin was present, generally in moderate amount, but varying from a faint trace to as high as 6 per cent. In the cases in which repeated examinations were made, the albumin was not always present but showed exacerbations and remissions. It was sometimes present at the onset, clearing up as the patient improved and reappearing later. In other cases it was not present until near the end. In fourteen cases, casts were present. These were generally of the hyaline and granular variety, but occasionally they were epithelial. The amount of the urine was reduced in nine cases and in a like number blood was present. In one case there was a faint trace of sugar found. In one of our cases the phenolphthalein output for two hours was too small to estimate on two occasions. The necropsy showed a very severe glomerulonephritis. In the other case the test was not done. In two cases there was infarction of the vessels of the testis.

Nervous System: The following symptoms were noted: Headache, 6; disturbances of sensation, 6; palsies, 4; convulsions, 4; visual disturbances, 3. In one case in which lumbar puncture was done the spinal fluid was sterile and in one of our cases the urea content was 0.323 per cent. In one of the cases showing visual disturbances a lesion was found in one of the retinal arteries. In the other two cases the retina was not examined post mortem.

Pathology.—The lesions of periarteritis nodosa consist in the peculiar arterial nodules and aneurysms and the changes in the various organs dependent on an insufficient or completely obliterated blood-supply. The larger arteries are not affected, the changes being limited to those of the size of the coronary and smaller. The veins and capillaries have never been found affected. The distribution of the lesions is practically that of the small and medium-sized arteries, that is, any arterial area may be involved. There are certain peculiarities, however, in that the arteries of the brain and spinal cord and the pulmonics are very rarely affected. Changes of the former have been found only four times and of the latter only twice. In some of the cases the central nervous system has not been examined. While the pulmonary arteries almost regularly escape, it is not at all uncommon to find the lesion in the bronchial arteries. The lesion has been found in practically every region of the body, including the retina, arteries to the nerves, the muscles, subcutaneous tissue, spermatic cord, diaphragm, etc. In nearly every case there are lesions in the heart, gastro-intestinal tract and kidneys. In 1905, Mönckeberg gave a table showing the site of the lesions in all the cases published up to that time. It does not seem necessary to enlarge on this table at present, especially as in many of the cases complete examinations were not made and all of the vascular areas were not investigated. Consequently, a table gives a rather erroneous impression.

Two important facts should be borne in mind. In the first place, in no less than five cases, no changes were noted in the arteries at the necropsy and the disease was not suspected until demonstrated in the sections. It is therefore possible that some cases may be overlooked both clinically and pathologically. That this is not often the case and also that the disease is in reality quite rare is shown by the fact that in a recent review of the sections of all cases of nephritis occurring at the Presbyterian Hospital during the past six years, no case of periarteritis nodosa was found, although the disease was carefully looked for.

In the second place, in five cases, the disease was limited to a relatively small vascular area or to one organ. Some time ago this was used as an argument against such a case being truly periarteritis nodosa, but it is now well recognized that such limitation may occur.

The gross appearance varies somewhat. In most cases there are both solid nodules and aneurysms. These occur with especial frequency at the points of branching, the size of the nodules having no relation to the size of the artery. The nodules are whitish to yellowish-white, moderately firm, later quite firm, and vary in size from those just visible and resembling miliary tubercles to those the size of a pea or slightly larger. They are generally situated at one side of the artery, but at times the whole wall of the artery seems thickened. As indicated above, many of the lesions are quite invisible to the naked eye. On section, they appear homogeneous, yellowish-white, and often show an associated occlusion of the artery. The larger nodules are generally aneurysmal dilatations, are generally softer in consistency and vary from a yellowish-white to a grayish-red or dark-red. These are almost regularly filled with thrombus and may slightly exceed a hazel-nut in size. The aneurysms and nodules may occur together on the same artery and often occur in thickly set bunches or are strung along the branch, giving it a corded or nodular feel. There may be petechial hemorrhages over the lesions and hemorrhagic extravasations into the surrounding tissues. The aneurysms may rupture resulting in hematomata. Quite regularly one finds infarcted areas in some of the organs from occlusion of the arteries at the site of the lesion. The portions of the arteries in which there are no nodules show no changes. The lesion may be quite early in one region and more advanced in another.

Perforation of the intestine may occur through an ulcer caused by infarction.

Microscopic Findings.—One of the chief points of controversy has been the question as to which coat of the artery is the seat of the primary change. The majority of observers believe that the disease begins in the adventitia, a fair proportion are equally insistent that the media represents the primary change. While there have been a few exponents of a primary intimal inflammation, all recent observers are agreed that the process does not begin there. It is believed that the injurious agent probably enters by way of the vasa vasorum or possibly the perivascular lymphatics, and affects one or other of the two outer coats. The reason that there has been so much difference of opinion concerning the relative degree of involvement of the different coats is that the lesion varies considerably in various portions of the same nodule and in the early and late stages of the disease. It is only by the study of serial sections and a review of the various histologic descriptions in the different cases that one can obtain a proper idea of the whole picture. Summing up these changes, we find the picture in the various coats as follows:

Intima: Is frequently not involved. No one has found changes here without simultaneous changes in the other coats. It is on this basis especially that the intima has been ruled out as the site of primary change. As a rule, with the changes in the other coats there is some degree of involvement of the intima. At times there is simply a proliferation of the endothelial cells, at times this is sufficiently marked to cause narrowing of the lumen. The cells then often appear large and polygonal in shape with clear, spindle-shaped or irregular nuclei; the tissue appears vacuolated; there may be some edema and fibrin with an occasional polymorphonuclear leukocyte and wandering cell and some nuclear fragments. A little later fibroblasts appear and with the formation of thrombi these cells grow into and organize the thrombus mass, at times leading to complete fibrous occlusion of the vessel. The changes may affect only a portion of the circumference or the whole intima. The cellular exudate of the media and adventitia may break through the internal elastica and invade the intima. The intimal thickening may extend for some distance beyond the nodule.

Internal Elastica: Generally at the site of the lesion it is wanting. In some cases it is quite sharply broken, the ends tending to curl up; more often it fades away into the exudate of the other coats. Often one can find it quite intact on the other side of the lumen. It may show definite small breaks in continuity in this region. At times it appears distinctly swollen at the site of the nodule.

Homogeneous Hyaline Ring: This is a very characteristic peculiarity of the lesion and consists of a zone of varying width, of more or less homogeneous appearance which takes the eosin stain quite intensely. It is generally situated just external to the internal elastica which is often preserved. In some cases it has been described as lying internal to the elastica and, as mentioned in one of our cases, it may lie both external and internal to it. In the more advanced lesion the band or ring is hyaline and structureless in appearance; but it seems quite possible to trace its formation from the degenerated media. Certainly, in the early stages the inner portion of the media can be seen to be more or less degenerated, its elements fusing together and taking the eosin stain more intensely. At a little later stage one sees the hyaline ring in this location quite distinctly, but it is quite possible to make out the degenerating smooth muscle cells within it.

Media: This coat is almost regularly involved. Mönckeberg even goes so far as to say that when no changes are demonstrable they really exist and that our present means of examination are not delicate enough to make them out. The early changes consist of swelling of the muscle-cells with a loss of their nuclei. One then finds a beginning inflammatory exudate of polymorphonuclear leukocytes, wandering cells and eosinophils, increasing to an extensive similar exudate with some fibrin, edema, nuclear fragments and vacuolization with increasing degeneration of the media, the formation of the hyaline band and later the appearance of fibroblasts and the gradual replacement of the exudate by fibrous tissue. At times, as in the aneurysm formation, the media is greatly thinned. The external elastica shows changes comparable to the internal.

Adventitia: An increasing number of observers have found changes in this coat without the occurrence of recognizable lesions elsewhere in the arterial wall. The earliest change consists of a small periarterial or adventitial collection of mononuclear wandering cells, polymorphonuclear leukocytes and eosinophils. I have been rather impressed with the occurrence of the eosinophils. It has been recognized for a considerable time that they are numerous in the lesions. In our sections, they seem attracted to the region of the arteries even when these show no change. The earliest lesion seen in our sections was in a small arteriole of the stomach-wall. This showed a small unilateral periarterial collection of cells almost all of which were eosinophils. With the older lesions the infiltration of cells increases until the adventitia becomes several times its normal width and merges imperceptibly with the periarterial infiltration which may extend for some distance into the surrounding tissue. The adventitia shows a beginning degeneration and then goes on to the formation of granulation tissue and finally fibrous tissue. At times one finds the perivascular lymphatics filled with an exudate similar to that described, never, however, to the same extent as seen in such streptococcus infections as erysipelas. The lesions do not go on to suppuration, and in this respect resemble the lesions in the organs in *Streptococcus viridans* endocarditis.

The lesion bears no relation to the supra-arterial epicardial fibroid nodules described among others by Knox. On gross examination these nodules are somewhat suggestive of periarteritis nodosa, but their histologic structure is quite different.

Brief mention has been made above to the occurrence of a similar disease in stags. Lüpke first described this condition in 1906. There has been some divergence of opinion as to the exact relationship between the two conditions. The descriptions of the lesion are rather brief and as we have had no opportunity of seeing sections taken in the cases we feel in no position to judge of their similarity. The consensus of opinion seems to be that the two conditions are very similar if not identical.

Duration.—The most acute case resulted in death in ten days. One case was followed for two years and ten months and was, as far as I have been able to discover, lost sight of at the end of that time. In general, the course is relatively rapid, 71 per cent. of the cases resulting fatally inside of three months. The duration is not always accurately given and in the following table I have considered only the period of acute symptoms.

Duration	No. of Cases
Not given	3
Less than 1 month	5
1-2 months	10
2-3 months	10
3-4 months	5
6-7 months	1
7-8 months	1
8-9 months	1
Over 1 year	2

Prognosis.—This is bad. Only one case in the series did not result fatally. This patient was followed for nearly three years and was alive at the end of that time. One patient in whom the diagnosis was made during life by the examination of an excised nodule was apparently cured of the disease, but died two years later of portal thrombosis. The necropsy showed no evidence of periarteritis nodosa except some small fibroid foci in close relation to the arteries. These were considered healed nodules. Other cases have shown similar but less marked tendency to healing in some regions, but always with progression of the process elsewhere.

Diagnosis.—One need but glance at the following list of diagnoses which have been made in the foregoing cases to obtain some idea of the puzzling nature of the disease: nephritis 7, meningitis 3, general miliary tuberculosis 2, general peritonitis 2, sepsis 2, dysentery, trichiniasis, morbus maculosus Werhlofii, encephalitis, typhoid, polymyositis, polyneuritis, dermatomyositis, cerebral hemorrhage, appendicitis and Schönlein's purpura rheumatica. One thing which one notices about these diagnoses, in reading over the cases, is that they were not considered quite typical and that they did not fully explain the picture.

While some few observers are inclined to the belief that the disease is not a distinct entity, but that it is merely an unusual incident in the course of some acute infectious disease, the majority of those who have studied it clinically and pathologically have come to the conclusion that periarteritis nodosa is a disease *sui generis*. Until the etiology is cleared up this point cannot be definitely determined; but the facts seem to point to the latter point of view.

The difficulty in arriving at a symptom-complex on which a diagnosis may be made lies in the wide-spread distribution of the lesions throughout the arterial system, with the corresponding symptoms in one or more regions or organs. Add to this the tendency of the disease to spread to new vascular areas and the occurrence of numerous overshadowing complications and one can appreciate the difficulties in the way of reaching a correct diagnosis.

Is it, then, possible to construct a symptom-complex of periarteritis nodosa sufficiently definite to make a diagnosis possible? Such a diagnosis has been correctly made only twice during life and then by the excision and examination of the subcutaneous nodules. Barring the presence of these nodules, an answer to the foregoing question must be in the negative. I believe, however, that a consideration of certain symptoms and groups of symptoms should make it possible to suspect the disease and should lead to more careful examinations for subcutaneous nodules. When the disease is once suspected every possible examination should be made to aid in clearing up the etiology. Since the occurrence of the above cases in the hospital, there have been at least two cases in which periarteritis nodosa has been suspected. In both of these there was a purpuric eruption and in both some of the spots were excised for microscopic examination. Neither case turned out to be an instance of this affection.

In practically all of the cases there is fever of a more or less irregular type, rapid pulse, often disproportionately so, anemia and progressive weakness and a striking leukocytosis with a high percentage of polymorphonuclears and in some, at any rate, a distinct eosinophilia. It is most important to have this eosinophilia confirmed by future observations, for if it be at all constant it will furnish a most valuable means of diagnosis. The above facts stand out clearly and represent a pretty definite background against which one must form the clinical picture. Of almost equal importance, even if not of quite as common occurrence, is the presence of edema and the symptoms of nephritis. While one would, of course, hesitate in making any diagnosis on these symptoms one should be equally reluctant in suspecting periarteritis nodosa without them. If one finds, in addition, some of the following less common but quite frequently present symptoms, it seems justifiable to suspect the disease: pains of tearing character in the extremities; pain and swelling of various joints; attacks of acute abdominal pain, colicky in character and generally located in the upper abdomen; vomiting; hemorrhagic eruptions; cyanosis; asthma. If subcutaneous nodules appear, the diagnosis can be easily settled by excision and histologic examination. In addition it should be remembered that the course of the disease is relatively short and that there may be distinct periods of remission of symptoms and improvement.

OTHER DOUBTFUL CASES

In addition to the list of cases considered in the preceding review, there are a few which have many points in common with periarteritis nodosa and which have been included in reviews of the disease by some authors. Some of them are possibly and even probably instances of this affection, but the failure of histologic examinations makes a decision impossible. The authors of these cases with the dates of publication are the following: Gee, 1871; Thompson, 1877; Sabin, 1901. In addition, there are the cases of Matani, Michaelis and Pelletan already referred to. The cases of Geipel and Eichorst are apparently not cases of this affection.

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The following references to the true cases of periarteritis nodosa are arranged alphabetically:

Beattie and Douglas: Jour. Path. and Bacteriol., 1912-13, xvii, 194.

Beitzke: Virchows Arch. f. path. Anat., 1910, cxcix, 214; Verhandl. d. deutsch. path. Gesellsch., 1909, xiii, 213.

Benda: Berl. klin. Wchnschr., 1908, xlv, 353.

Benedict: Ztschr. f. klin. Med., 1907, lxiv, 405.

Bomhard: Virchows Arch. f. path. Anat., 1908, cxcii, 305.

Chvostek and Weichselbaum: Allg. wien. med. Ztg., 1877, xxviii, 257.

Cooke: Proc. Path. Soc., Philadelphia, 1911, New Series, xiv, 96.

Datnowski: Wien. klin. Rundschau, 1911, xxv, 469, 488, 503, 520; Inaug. Diss., Berl., 1909; Ueber Periarteritis nodosa, E. Ebering, Berlin, 1909.

Dickson: Jour. Path. and Bacteriol., 1908, xii, 31.

Eppinger: Arch. f. klin. Chir., 1887, xxxv, Sup., p. 126.

Ferrari: Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1903, xxxiv, 350.

Fletcher: Ibid., 1892, xi, 323.

Freund: Deutsch. Arch. f. klin. Med., 1899, lxii, 537.

Graf: Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1896, xix, 181.

Hart: Berl. klin. Wchnschr., 1908, xlv, 1305; Veröffentl. d. Hufeland Gesellch., Berlin, 1909, ii, Teil 7-18.

Jonas: Munchen. med. Wchnschr., 1912, dxcii, 1685.

Krzyszkowski: Quoted by Ferrari, Przeglad. Lekarski, 1899.

Künne: Frank. Ztschr. f. Path., Wies., 1910, v, 107; Dissert. Leipsic, 1909.

Kussmaul and Maier: Deutsch. f. klin. Med., 1866, i, 484.

Lewis: Proc. Path. Soc., Philadelphia, 1912, New Series, xiv, 134.

Longcope: Bull. Ayer Clin. Lab., Pennsylvania, Hosp., 1908, v, 1.

Meyer: Virchows Arch. f. path. Anat., 1878, lxxiv, 277.

Mönckeberg: Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1905, xxxviii, 101; Verhandl. d. deutsch. path. Gesellsch., 1909, xiii, 213.

Moriani: Inst. d'anat. Path., Sienne, 1910; abstr. Arch. d. mal. d. coeur., 1911, iv, 460.

Müller: Festschr. z. Feier d. 50 jahr. Bestch. d. Stadtkrankenhaus z. Dresden, 1899, p. 457.

Oberndorfer: München. med. Wchnschr., 1907, lii, 2618; Berl. klin. Wchnschr., 1907, xlvi, 1496.

Rokitansky: Quoted by Eppinger, Denkschr. d. kaiserl. Akad. d. Wissensch., Wien, 1852, iv, 49.

Rosenblath: Ztschr. f. klin. Med., 1897, xxxiii, 547.

Schmidt: Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1908, xliii, 455.

Schmorl: Verhandl. d. deutsch. path. Gesellsch., 1903, 203.

Schreiber: Inaug. Diss., Königsberg, 1904.

Versé: Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1906, xl, 409.

Veszprémi and Jancsó: Beitrage, Ibid., 1903, xxxiv, 1.

Veszprémi: Ibid., 1912, lii, 476.

Von Kahlden: Ibid., 1894, xv, 581.

Zimmermann: Arch. d. Heilk. (Wagner), 1874, xv, 167.

The following is a list of the literature on the other cases mentioned and other articles dealing with the disease:

Eichorst: Deutsch. Arch. f. klin. Med., 1904, lxxx, 75.

Gee: St. Bartholomew's Hosp. Rep., 1871, vii, 147.

Geipel: Munchen. med. Wchnschr., 1907, xx, 1057.

Jaeger: Virchows Arch. f. path. Anat., 1909, cxcvii, 71; XIII, Verhandl. d. deutsch. path. Gesellsch., Leipsic, 1909, 209.

Lüpke: Verhandl. d. deutsch. path. Gesellsch., Stuttgart, 1906, 149.

Matani: De aneurysmatis praecordiorem morbis, Florenze, 1755.

Michaelis: Quoted by Eppinger. No reference.

Pelletan: Clin. Chir. on Memoires et observations de chir. clin. et sur d'autres Objects, etc., Paris, 1810, T. I.

Pertik: Centralbl. f. allg. Path. u. path. Anat., 1908, xix, 90.

Sabin: Bull. Johns Hopkins Hosp., 1901, xii, 195.

Thompson: Med. Times and Gaz., 1877, 56.

Thorel: Ergebn. d. Path. (Lubarsch and Ostertag), 1910, ii, 528.

Knox: Supra-arterial Epicardial Fibroid Nodules, Jour. Exper. Med., 1899, iv, 245.

LATENT HYPERTROPHY OF THE HEART IN THE NEPHRITIS OF CHILDREN *

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Ever since Traube established the close relationship between chronic nephritis and cardiac hypertrophy and the recognition of arterial hypertension as the cause of the latter, there has been an increasing tendency to see in a left ventricular hypertrophy presumptive evidence of an underlying chronic nephritis, in the absence of some other cause to explain it.

Whether we follow the school of Romberg, which makes arterial hypertension the consequence of a glomerular lesion, or adhere to the opinion of Jores,[1] that high pressure results from a fibrosis of the smaller vessels, including those of the kidney, it seems agreed that the occurrence of high blood-pressure and its corollary, cardiac hypertrophy, point to a lesion in the vascular component of the kidney.

It is, however, not only in the outspoken cases of nephritis that we expect to find cardiovascular changes. Even in those not uncommon cases of nephritis in which evidences of renal insufficiency and anomalies of the urinary secretion are negligible or absent, heart changes are frequently encountered.

So true is this, that very often, chronic nephritis in adults, in so far as it affects the functions of any organs and the health of the individual, is essentially a cardiovascular disease and death results from heart failure.

While these facts are well recognized in the case of adults, the rôle which cardiac hypertrophy plays in the nephritis of children is not so well known. Various difficulties surround the solution of this question. The nephritis of children is in many cases not a frank one and may be difficult of diagnosis. This is especially true of paedonephritis, a condition the recognition of which we owe to Heubner.[2] He has directed attention to the frequency of very mild cases of chronic nephritis in children, in which the urinary signs may consist only in an albuminuria, at times intermittent, with occasional casts. These signs are easily overlooked in a cursory examination, especially as manifestations of renal inadequacy are frequently absent. To the question, "Does

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* Submitted for publication, Jan. 14, 1914.

[1] Jores: Deutsch. Arch. f. klin. Med., 1908.

[2] Heubner: Jahrb. f. Kinderh., 1913.

cardiac hypertrophy complicate such cases in children?" we can give only a doubtful and clinical answer, because autopsies are rarely available to instruct us, due to the benign character of the disease. We are dependent, therefore, in deciding this question on such aids as clinical examination can afford us. These are, examination of the heart by the usual methods and estimations of the blood-pressure.

To take up the latter first, it must be confessed that the evidence of increased blood-pressure in children is equivocal. In them, the normal variations are considerable and it is a matter of difficulty to decide in the individual case whether the pressure has exceeded its physiological limits. On the other hand, the clinical signs on which we are accustomed to base a diagnosis of hypertrophy of the left ventricle are more determinate. They are as follows: a forcible apical impulse often heaving in character; corresponding to this there is a loud booming first sound of so-called good muscular quality, which may, in pronounced cases, be described as crunching. Occasionally this sound is roughened or split so that it may simulate a short presystolic murmur. There is always an accentuation of the second aortic sound. While these changes in the cardiac physical signs are easily elicited in the larger hearts of adults, in the case of children with moderate grades of hypertrophy they may not be so striking and may be masked by the cardiac overaction consequent on the examination.

The necessity for the determination of these secondary heart changes becomes the greater when it is realized that such information must be of great value in guiding prognosis; for setting aside for the moment the question of cure in mild cases of chronic nephritis, the possibility of complete recovery of the patient will be determined to a great extent by the degree to which the renal injury has compromised the efficiency of the cardiovascular system.

In the solution of this question, the fluoroscope can afford us decisive help. It is well known through the work of Dietlen,[3] Grodel[4] and Otten,[5] among others, that alterations in the mass of the heart and in the volume of its cavities result in typical changes in its morphology, varying with the site of the lesion. Of these changes none is more characteristic than hypertrophy or dilatation of the left ventricle. An increase in the size of this structure whether of mass or volume, causes the left border of heart as seen on the screen to acquire a more or less elliptical curve, its apex becomes blunt and rounded and its axis approaches the horizontal. Very striking is the deliberate, forcible and slow contraction of the left ventricle, of unusual amplitude. Not uncommonly, under the influence of increased stroke-volume or because the blood is ejected with greater force, the aorta becomes dilated and shows an exaggerated expansile pulsation with each systole (Fig. 1).

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[3] Dietlen: Deutsch. Arch. f. klin. Med., 1903, No. 88.

[4] Groedel: Deutsch. Arch. f. klin. Med., 1908, No. 93.

[5] Otten: Deutsch. Arch. f. klin. Med., 1910, No. 105.

The change in the shape of the heart here described occurs irrespective of any increase in its size to percussion and admits of the recognition of slight degrees of hypertrophy, which in many cases must be difficult or altogether impossible by the ordinary methods of examination.

In the application of fluoroscopy to the determination of heart changes in nephritic children, I have studied different varieties of the disease. In some cases, children whose urines on one examination were negative were nevertheless included in the series because they had had scarlet fever with or without acute nephritis and because subjective cardiac symptoms suggested the possibility of cardiac involvement. In these cases it was usually possible by the orthodiagraph to demonstrate the characteristic heart appearance above described. I have especially chosen cases in which on physical examination alone, owing to absence of enlargement of the heart and because of the trivial character of the subjective symptoms, cardiac disease was difficult of proof and in which for this reason the help afforded by fluoroscopy was all the more pronounced. In a similar manner, a number of the more severe and outspoken forms of chronic nephritis with marked glomerular lesions was studied; finally a lesser number of acute nephritides was examined to ascertain, if possible, the occurrence of hypertrophy of the heart in this condition.

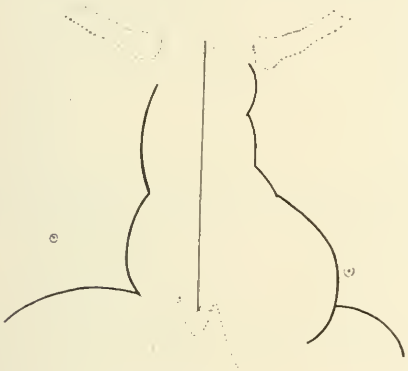

Figure 1

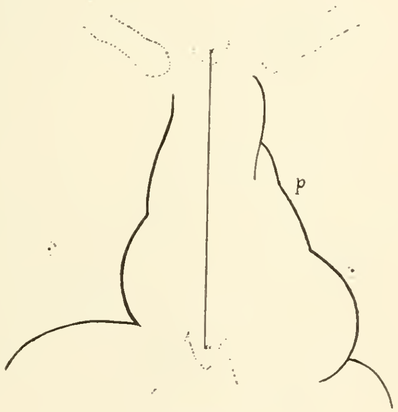

Figure 2

Fig. 1.—Left ventricular hypertrophy.
Fig. 2.—Left ventricular hypertrophy with secondary dilatation; mitralization.

TABLE 1.—Cases—

Case No.	Age Years	Etiology of Nephritis	Present Nephritic Symptoms	Previous Nephritic Symptoms
1	5	Sarlet fever 3 months ago.	Pale urine; faint trace albumin.	Three months ago general edema; vomiting; albumin, ++.
2	12	Scarlet fever 8 years ago.	Urine negative.	None.
3	11	Scarlet fever years ago.	Pale, pigmentation; headache; albumin, trace.	Headache for some years.
4	14	Measles and diphtheria in childhood.	Pale; puffy eyes; urine: trace of albumin and hyaline casts; bl. pr. 125.	None.
5	10 6/12	Scarlet fever 1 year 8 months ago.	Pale pigmented skin; trace of albumin; hgb., 80 per cent.	For 1 yr. 8 mo. has been pale; headache; persistent albuminuria; no casts.
6	5	Tonsillitis years ago; severe tonsillitis 3 months ago.	Tired; poor appetite; pigmentation of skin; albumin, ++; hyaline casts, sp. gr., 1.012.	One month ago, albumin found in urine.
7	9	None ascertained.	Headache; urine negative.	Ten months ago, general edema; headache; bloody urine (acute nephritis).
8	9	Scarlet fever 8 months ago.	Pale; puffy eyes; urine negative.	Eight months ago, edema and hematuria.

—OF MILD NEPHRITIS

Cardiac Symptoms	Cardiac Signs	Heart Size, cm.		Heart Shape and Action
			Normal	
None.	Forcible apex beat; impure first sound; aortic second accentuated.	Ml. .... 6.3 Mr. ...... 3.2 Tr. .... 9.5 L. 9.4	Ml. .... 5.92 Mr. .... 2.85 Tr. .... 8.77 L. 9.3	Hypertrophied left ventricle; aorta wide; slow, deliberate, strong contraction.
....	Apex, 1 finger outside nipple; forcible apex beat; slow; aortic second accentuated.	Ml. .... 6.9 Mr. ...... 4.5 Tr. ...... 11.4 L. 12.0	Ml. .... 7.2 Mr. ...... 3.1 Tr. ....10.3 L. 11.1	Left ventricular hypertrophy; dilated aorta; slow, forcible action.
None.	Heaving apical impulse; faint systolic murmur; accentuated aortic sound.	Ml. .... 7.0 Mr. ...... 3.1 Tr. ....10.1 L. 9.9	Ml. .... 6.3 Mr. .... 3.04 Tr. .... 9.4 L. 10.1	Hypertrophy with secondary dilatation of left ventricle; mitralization; strong contraction; aorta much dilated.
Palpitation; dyspnea; cough; pallor.	Apical sound booming; split; pseudostenotic murmur; aortic second +.	Ml. .... 6.9 Mr. .... 3.0 Tr. .... 9.9 L. 10.0	Ml. .... 7.28 Mr. .... 3.11 Tr. ....10.39 L. 11.1	Typical left ventricular hypertrophy; strong contraction; aorta dilated.
Pale, weak, decompensated, dyspnea; after 2 weeks' treatment no symptoms.	Heart dilated; apex impulse, diffuse, thrusting, apical, systolic murmur, P_2++, A_2+; poor pulse.	Ml. .... 7.1 Mr. .... 3.7 Tr. .... 10.8 2 weeks later Ml. .... 7.3 Mr. ...... 3.2 Tr. 10.5	Ml. .... 6.35 Mr. ...... 3.04 Tr. .... 9.4 L. 10.1	Dilated and hypertrophy of left ventricle; mitralization; poor contraction.
Easily tired; palpitation.	First sound good muscular quality; A_2 ringing.	Ml. .... 5.6 Mr. .... 3.2 Tr. .... 8.8 L. 8.5	Ml. .... 5.7 Mr. .... 2.8 Tr. .... 8.5 L. 9.3	Hypertrophy of left ventricle; deliberate, strong contraction; wide aorta.
Dyspnea and palpitation on exertion.	Forcible apex beat; first sound good muscular quality; A_2 ringing.	Ml. .... 6.5 Mr. ....4.1 Tr. ...... 10.6 L. 11.0	Ml. .... 6.79 Mr. ...... 3.08 Tr. .... 9.8 L. 10.9	Hypertrophy of left ventricle; enlargement to right; forcible pulsation of left ventricle and aorta.
Dyspnea and palpitation on exertion.	First sound impure; basic sounds accentuated; A_2 ringing.	Ml. .... 6.5 Mr. .... 3.5 Tr. ....10.0 L. 10.3	Ml. .... 5.97 Mr. .... 2.8 Tr. .... 8.8 L. 9.3	Left ventricle hypertrophied and dilated; mitralization; aorta dilated.

I. CASES OF MILD NEPHRITIS

In this group are included eight cases the findings in which are summarized in Chart 1. A résumé of this shows the following: five had scarlet fever from three months to eight years previously. Five have a history of a previous acute nephritis, which was in all except one of the glomerular type. Five of them at present have mild cardiac symptoms, such as dyspnea on exertion and palpitation. So mild were these complaints that often they were not commented on by the patient and had to be elicited by questioning.

Of these eight cases, five showed albumin in the urine usually in traces, not constantly, and with the occasional appearance of hyaline casts. In three of them, on a single examination, albumin was not found.

The physical signs pointing to cardiac hypertrophy were the classical ones. They were in evidence in each case and especially significant was the clear, and at times ringing aortic second sound, which usually exceeded in intensity the pulmonic sound. This phenomenon, reversing as it does the condition normally found in children, is very striking.

In all the cases in this group the orthodiagraphic examination disclosed a more or less marked hypertrophy of the left ventricle, which was further manifested by a slow deliberate ample contraction of this structure. The aorta was frequently dilated and showed an unusually marked pulsation. It will be observed on comparing the size of the heart with the normal control figures (Veith[6]), that four of them showed a moderate dilatation to the left, too small, however, to be demonstrated by percussion.

There was evidence in two of the patients of a muscular insufficiency of the left ventricle. This gave rise in Case 5 to symptoms of decompensation together with a systolic murmur at the apex and some impurity of the first sound. That this cardiac weakness, with the accompanying murmur, took its origin in a dilatation of a previously hypertrophied ventricle rather than in an organically diseased valve is evident from a study of the orthodiagrams in this case. This murmur also was not present on examination a year before.

Figure 2 is the orthodiagram made Jan. 22, 1913, when the patient was decompensated. The apical impulse was powerful, though diffuse, and both basic sounds were accentuated, the pulmonic more so than the aortic. The systolic murmur was loud and was transmitted to the axilla. The tracing of the heart exhibits a hypertrophy and dilatation of the left ventricle, the left border extending beyond the nipple. The pulmonary artery and the left auricle (p) owing to the regurgitation from the mitral valve, have also become dilated. The appearance here described differs radically from that seen in mitral insufficiency resulting from imperfect valves, as will be evidenced from a study of Figure 3. The vertical position, the shape of the left ventricle, the increase in size to the right and the smallness of the aorta combine to create a heart silhoutte different from the preceding and just as distinctive. This patient was treated with rest and digitalis for one week with a complete subsidence of all symptoms. At that time on examination, the first sound was clear and the murmur was scarcely audible; the second aortic sound was accentuated and was louder than the pulmonic. The orthodiagram showed the heart to be practically of the same size as previously; the pulmonary artery and the left auricle, however, were more prominent, indicating probably a permanent backflow from the ventricle. This condition, so-called mitralization, was present in a few of the cases and confirmed the findings of physical examination.

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[6] Veith: Jahrb. f. Kinderh., 1908, No. 68.

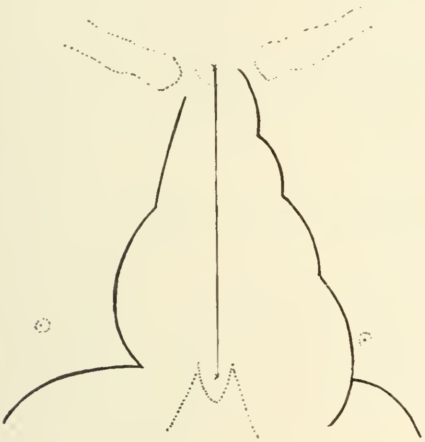
Figure 3

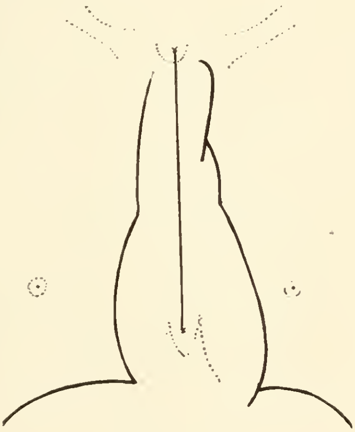
Figure 4

Fig. 3.—Valvular mitral insufficiency.
Fig. 4.—Drop heart.

In this series of cases the absence of urinary evidence in three of the cases of nephritis may be the occasion of some criticism and may be taken as a cause for doubting the existence of an actual nephritis in them. In this regard, it should be stated that only one urinary examination was possible usually; and when it is remembered that cases with mild nephritis may have an albuminuria only intermittently, it is not surprising that it should have been missed on a single test. Two of these three cases, however, are known to have had an acute hemorrhagic nephritis, one of them eight and the other ten months previous to this report, a fact which greatly diminishes the importance of the negative urinary finding. In the absence of some other cause to explain the left ventricular hypertrophy, I believe, when due weight is given to the etiology, that a diagnosis of a latent nephritis in these cases has at least a presumptive basis. It is precisely because the fluoroscope is capable of disclosing a typical change in the configuration of the heart that it is of value, as from it we are permitted to infer the presence of a renal lesion, which at the moment is producing no anomaly in the

TABLE 2.—Cases of—

Case No.	Years Age	Etiology of Nephritis	Present Nephritic Symptoms	Previous Nephritic Symptoms
1	10	Not ascertained	Pale, puffy face; headache; albumin +; hyaline and granular casts and red cells.	Five weeks ago, acute nephritis; edema, hematuria.
2	2 9/12	Scarlet fever three months ago.	Urine shows hyaline and granular casts and red cells.	Three months ago had acute glomerular nephritis with edema.
3	18	Scarlet fever ten years ago.	Edema of eyes; urine: albumin and casts.	Edema of face eight months; albumin and casts.
4	14	Not ascertained	Pale, puffy face; general edema; albumin, casts and red blood cells; bl. pr. 130.	Similar symptoms months ago.
5	16	Scarlet fever thirteen years ago.	Pale; urine: albumin ++, many hyaline and granular casts; red cells; edema of face and feet.	Similar symptoms for 7 weeks.
6	7	Measles (?)	General edema; albumin +++; hyaline and granular casts and red cells.	Eleven weeks ago headache and edema up to present.

urinary secretion. An analogy may be seen in the condition of presclerosis of adults in whom the rise of blood-pressure may be insignificant and the urinary changes at times absent, but in whom Pal could usually demonstrate a cardiac hypertrophy.

—Severe Nephritis

Cardiac Symptoms	Cardiac Signs	Heart Size, cm.		Heart Shape and Action
			Normal	
Palpitation and dyspnea on exertion.	Apex outside nipple; first sound impure; strong impulse; A_2++; thick arteries.	Ml. 7.7 Mr. 2.8 Tr. 10.5 L. 10.0	Ml. 5.97 Mr. 2.8 Tr. 8.2 L. 9.3	Normal action; heart dilated to left; no distinct evidence of hypertrophy.
None.	First sound accentuated; A_2 accentuated.	Ml. 4.5 Mr. 3.1 Tr. 7.6 L. 7.2	Ml. 5.4 Mr. 2.2 Tr. 7.6 L. 8.6	Forcible action; left ventricular hypertrophy.
None.	Evidence of hypertrophy of left ventricle.	Ml. 8.4 Mr. 4.6 Tr. 13.0 L. 12.5	Ml. 8.0 Mr. 3.8 Tr. 11.8 L. 13.0	Typical hypertrophy of left ventricle with slight dilatation.
None.	Left ventricular hypertrophy; split first sound; pseudo mitral stenosis.	Ml. 9.1 Mr. 3.9 Tr. 13.0 L. 12.7	Ml. 7.28 Mr. 3.1 Tr. 10.39 L. 11.8	Hypertrophy and dilatation of left ventricle; aorta dilated; forcible action.
Dyspnea and palpitation on exertion.	First sound booming; A_2 + + +; bl. pr. 130; forcible impulse.	Ml. 8.2 Mr. 4.5 Tr. 12.7 L. 12.7	Ml. 7.9 Mr. 4.3 Tr. 12.4 L. 13.1	Action, forcible, deliberate; hypertrophied left ventricle.
None.	First sound booming; basic sounds, especially aortic accentuated.	Ml. 5.9 Mr. 2.8 Tr. 8.7 L. 9.3	Ml. 5.7 Mr. 2.8 Tr. 8.5 L. 9.3	Shape of hypertrophy; aorta wide and shows marked pulsation.

II. CASES OF SEVERE NEPHRITIS

This group comprises six cases of chronic and subacute hemorrhagic nephritis in children (Chart 2). The etiology in three of these was scarlet fever; it was undetermined in the others. With one exception, these patients had an acute hemorrhagic nephritis with edema, a relatively short time previously, signs of which continued with abated severity to the present. The urines of all of them contain large amounts of albumin, hyaline and granular casts and red blood-cells. Of cardiac symptoms, these patients had few, only two of them complaining of dyspnea on exertion. The physical signs were uniformly those of hypertrophy of the left ventricle without dilatation, findings which were convincingly shown by orthodiagraphic tracings. These in every case showed the typical appearance I have described, associated with some aortic dilatation. It should be stated that the blood-pressure readings were not sufficiently elevated to permit any deductions to be made from them.

TABLE 3.—Cases of—

Case No.	Age Years	Etiology of Nephritis	Present Nephritic Symptoms	Previous Nephritic Symptoms
1	9	Tonsillitis 4 days ago.	Edema of face and legs; albumin ++; casts and red cells.	....
2	16	Severe tonsillitis 4 weeks ago.	Pale, general edema, ascites; albumin ++; casts and red cells.	Ten days ago got edema, headache, vomiting, oliguria.
3	14	Tonsillitis 4 weeks ago.	Pale, general edema; hydrothorax; albumin ++; casts and red cells; bl. pr. 164.	Three and one-half weeks ago had hematuria; vomited.

III. ACUTE GLOMERULAR NEPHRITIS

This group comprises three cases of acute nephritis occurring in children, with a duration at the time of the examination of four days in the first, ten days in the second and four weeks in the third, and caused in each case by an acute tonsillitis. Apparently, in the first case, sufficient time had not elapsed for the production of any alteration in the circulation, for neither physical signs nor the fluoroscope reveal any deviation from the normal. In the two remaining cases an exquisite left ventricular hypertrophy had developed with the usual clinical signs and fluoroscopic appearance.

It will thus appear that as early as the tenth day and the fourth week after the onset of an acute nephritis, the heart is already hypertrophied. That this is so should not be surprising when it is recalled that Butterman found a rise in blood-pressure as early as forty-eight hours after the beginning of a nephritis, and that "in primary nephritis the rise in blood-pressure is rarely missed" (Krehl[7]). It is natural to conceive that a hypertension lasting four weeks with the increased work this entails on the heart, should, after the manner of muscular tissues elsewhere in the body, lead to hypertrophy from overwork.

It should be emphasized that we are here not dealing with a simple dilatation of the heart, dependent on a myocarditis such as might be assumed to be present in a weakened and edematous patient. To make this more evident, in Case 2 the patient was examined on two occasions.

—ACUTE NEPHRITIS

Cardiac Symptoms	Cardiac Signs	Heart Size, cm.		Heart Shape and Action
			Normal	
None.	None.	Ml. .... 5.8 Mr. .... 3.7 Tr. .... 9.5 L. 9.7	Ml. ...... 6.35 Mr. ..... 3.06 Tr. .... 9.4 L. 10.1	Normal shape and action.
Palpitation on exertion.	Diffuse, forcible apex beat; first sound, booming; $A_2 + +$; bl. pr. 155.	Ml. .... 7.8 Mr. .... 5.8 Tr. ....13.6 L. 13.4	Ml. .... 7.9 Mr. .... 4.3 Tr. ....12.1 L. 13.1	Left ventricular hypertrophy; enlargement to the right.
None.	First sound booming; A_2+.	Ml. .... 7.8 Mr. .... 3.5 Tr. ....11.3 L. 12.0	Ml. .... 7.28 Mr. .... 3.1 Tr. ....10.39 L. 11.8	Left ventricular hypertrophy; slight enlargement to right; forcible action.

At the first examination ten days after the onset of the nephritis, there were generalized edema, a diffuse though powerful apical impulse and accentuation of both basic sounds. The orthodiagram at this time showed a hypertrophy of the left ventricle; in addition, enlargement of the pulmonary artery and increase of the heart diameter to the right. There was no systolic murmur, and these secondary changes may be looked on as caused by a muscular insufficiency of the left ventricle with a resulting mitralization. Two and one-half weeks later the urine had to a great extent cleared up, the edema had subsided and the patient felt quite well. The examination of the heart nevertheless disclosed outspoken signs of hypertrophy. The muscular weakness of

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7. Krehl: Nothnagel's System, 1912, xv.

the left ventricle, however, had mostly disappeared; the heart was less enlarged to the right and the pulmonary artery was much diminished in size.

I have attempted to show in a series of cases of acute and chronic nephritis in children that it was possible to demonstrate in most of them the presence of heart changes, which consist in a hypertrophy of the left ventricle associated at times with slight degrees of dilatation and in a few cases with evidences of enlargement of the right side of the heart. That such changes should occur in the outspoken cases of nephritis with edema and glomerular inflammation, though only weeks or months have elapsed, is perhaps to be expected; one may hesitate, however, in those cases of nephritis in which the urinary findings are often ambiguous and in which, therefore, one may assume the existence of only slight damage to the kidney, to believe that the structure of the heart has suffered alterations capable of recognition — alterations which in their importance overshadow the renal lesion.

The force of this objection is mitigated by the fact emphasized by Jores and Fisher[8] that there is not necessarily a quantitative relation between the degree of kidney disease and the degree of cardiac hypertrophy; similarly Pal,[9] in the presclerosis of Huchard, on autopsy found well developed cardiac hypertrophy, where only a slight grade of kidney inflammation was in evidence.

The frequent occurrence of scarlet fever in the cases here reported is in accord with what is well known in regard to the rôle which this disease plays in the causation of nephritis. All observers are not agreed, however, as to the frequency with which scarlet fever is responsible for chronic nephritis, especially of the milder forms. Thus Escherich and Schick[10] are inclined to doubt the frequent connection of scarlet fever with chronic nephritis. Rosenfeld,[11] on examining forty-one cases of scarlatinal nephritis at intervals of one to ten years, found an albuminuria which was spontaneous or orthostatic, associated in seven cases with casts. Stroink[12] believes that many contracted kidneys in young adults take their origin in an acute scarlatinal nephritis with a long intervening albuminuria, which is easily overlooked.

Heubner, especially, has associated scarlet fever with the milder forms of chronic nephritis in children. According to him, these patients go on for years with minor urinary signs; either they improve at the time of puberty, which seems to have a favorable influence on the nephritis, or they go on to contraction in later years.

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[8] Fischer: Deutsch. Arch. f. klin. Med., 1911, No. 109.

[9] Pal: Med. Klin., 1909.

[10] Escherich and Schick: Nothnagel's System, Supplement, p. 143.

[11] Rosenfeld: Ztschr. f. Kinderh., 1912.

[12] Stroink: Jahrb. f. Kinderh., 1911.

There seems to be less agreement as to the heart changes which may be a sequel of nephritis and scarlet fever. During the acute stage of scarlet fever according to Lederer and Stolte, evidences of heart-changes are present in as many as 70 per cent. of the cases. Friedlander,[13] in children who died of post-scarlatinal nephritis, found a distinct cardiac hypertrophy in nearly all cases, dependent apparently on the nephritis. Sörenson[14] similarly found a hypertrophy in acute nephritis. Baginski,[15] on the other hand, by clinical methods, found no evidence of cardiac hypertrophy in the acute nephritis of children. In the chronic albuminurias after scarlet fever, however, Rosenfeld rarely found heart-changes. A similar observation occurs in the reports of Stroink and Heubner. There is, nevertheless, *a priori*, reason to suppose that in at least some of these cases the conditions making for cardiac hypertrophy are operative. If it is true that a short time after the onset of an acute nephritis, the blood-pressure already shows an appreciable rise, with presumably its concomitant effect on the cardiac musculature, it is plausible to assume that a similar, though less severe kidney disturbance, will after being operative for months or years, give rise to analogous changes in heart structure.

In the decision of this question, as I have endeavored to show, the orthodiagram is of considerable help in that it supplies objective proof to support physical signs which ordinarily might leave us in doubt. This is especially of value in cases of orthostatic albuminuria, in which for a long time one may be unable to decide whether there is really a nephritis present or only a functional or postural albuminuria exists. Now in orthostatic albuminuria of children, subjective cardiac symptoms are at least as frequent as in the milder forms of chronic nephritis. In addition, in many cases, there are distinct evidences of cardiac overaction and relative cardiac insufficiency, to which such terms as "growth hypertrophy," "juvenile heart" and "dilatative heart weakness" have been applied.

Bass and Wessler,[16] in a study of a series of such cases have noted the great frequency of the asthenic type of heart, the so-called "drop-heart." Such hearts frequently give the clinical signs of hypertrophy; on orthodiagraphic examination, however, they are found, as a matter of fact, to be smaller than normal and present a unique appearance quite different from a heart of hypertrophy (Fig. 4).

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[13] Friedlander: Arch. f. Anat. u. Physiol., 1881.

[14] Sörenson: Ztschr. f. klin. Med., 1891.

[15] Baginski: Arch. f. Kinderh., 1902, No. 33.

[16] Bass and Wessler: THE ARCHIVES INT. MED., 1913, No. 11.

It will thus be seen that the presence of such a "drop-heart" in a case of albuminuria will favor the diagnosis of a functional condition and will justify a brighter prognosis; on the other hand, the finding of a left ventricular hypertrophy associated with an albuminuria will afford a basis for a diagnosis of an organic kidney disease.

122 West One Hundred and Eighteenth Street.

THE NON-SPECIFICITY OF CARBOHYDRATE TOLERANCE TESTS *

JOSEPH C. FRIEDMAN, M.D., AND SOLOMON STROUSE, M.D.
CHICAGO

About three years ago we commenced using carbohydrate tolerance tests as a routine procedure in all cases in which there might exist a functional disturbance of any of the ductless glands. At that time we thought that if the test were carefully worked out in a large enough series of cases, some diagnostic importance might be attached to it. From a knowledge of the work of previous investigators, we realized that sugar tolerance could never be a specific test, for at least in the metabolism of glucose many organs play a rôle. Von Noorden's[1] conception of the liver as the central figure in a circle of pressor and depressor influences seemed a logical working basis — hypophysis, thyroid and adrenal being pressors, and pancreas the depressor of carbohydrate metabolism. Clinical evidence was forthcoming to show that states of hyperacidity of the pressor organs and hypo-activity of the depressors are often associated with spontaneous glycosuria, or at least a lowered threshold for carbohydrates, whereas in hypo-activity of the pressors the tolerance for carbohydrates is increased.

Strauss'[2] levulose test for hepatic insufficiency, which was based on Sachs'[3] experiments with dehepatized frogs, had been hammered from all sides, with results which could only be classed as discordant. At the time we commenced our tests we thought that there were many factors to account for this, among which may be mentioned:

1. Lack of standard technic.
2. The difficulty of knowing what is meant by "hepatic insufficiency."
3. The probability that other organs played an important rôle in the utilization or non-utilization of sugars employed in the test.

After our actual work was finished, but before this article was written, appeared the excellent article by Bloomfield and Hurwitz[4]

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* Submitted for publication May 24, 1914.

* From the Department of Clinical Research, The Michael Reese Hospital, Chicago.

[1] Von Noorden: Med. Klinik, 1911, vii, 1.

[2] Strauss: Deutsch. med. Wchnschr., 1901, xxvii, 757.

[3] Sachs: Ztschr. f. klin. Med., 1899, xxxviii, 87.

[4] Bloomfield, A. L., and Hurwitz, S. H.: Tests for Hepatic Function: Clinical Use of the Carbohydrates, Johns Hopkins Hosp. Bull., 1913, xxiv, 375. Full review of the literature.

on the clinical use of carbohydrates as tests for hepatic function. These authors review critically the literature, discuss the theoretical basis for the application of sugar tolerance, and come to the conclusion that the inconclusive experience of others might be expected because "the theoretical basis for the use of sugars as tests for hepatic function is definitely deficient." From a practical standpoint, they maintain that difficulties must arise from (*a*) the method of administration; (*b*) faulty absorption; (*c*) portal obstruction; (*d*) retention of sugars in renal disturbances, and (*e*) the variable amounts of carbohydrate in the diet. A part of the summary of their work is worth quoting verbatim:

> A consideration of the extrahepatic factors involved in the sugar-regulating metabolism, the influence of the glands of internal secretion and of the vegetative nervous system, the ability of other tissues than the liver to handle sugar, and the ability of the uninjured liver substance to compensate in disease, make the sugars theoretically unsatisfactory as tests for hepatic insufficiency.
>
> There are serious objections to the methods as they have been applied, namely, the use of arbitrary amounts of sugar, and the use of a definite standard of excretion.

The main point we attempted to investigate was the relation between sugar tolerance and various clinical states involving one or more than one of the ductless glands. The first great practical difficulty was in the standardization of technic. How much sugar should be given? Is a quantitative determination of the amount excreted necessary? To adopt an arbitrary amount of sugar as a test for all individuals is obviously not a logical procedure, nor can the body weight be considered more logical as a standard of efficiency of sugar metabolism. Apparently the only scientific method is, as Bloomfield and Hurwitz point out, the impossible one of testing the tolerance before and after the disease has started. The practical solution was a compromise: If most normals do not excrete sugar on 100 gm. dextrose and do excrete sugar on 125 gm., then we may fix 100 gm. as the "normal" threshold of sugar tolerance. In our series of approximately 225 tests we included many normals, and as an arbitrary standard we generally started with 100 gm. dextrose, or 90 gm. levulose. The difficulties of administration were easily overcome with the smaller doses by embodying the sugar in 250 c.c. of *sour* lemonade given early in the morning, on an empty stomach. When the doses were larger we occasionally encountered nausea and vomiting, which naturally spoiled the test. In almost every case both dextrose and levulose were administered with *one* day intervening, and only in exceptional cases in which haste was demanded were the sugars given on succeeding days. The urine was collected just before the sugar was given, and at three intervals of two hours each after the administration of the sugar. Qualitative tests only were employed. When dextrose was given, reduction of Fehling's in a urine negative before the test was called "positive." With levulose we also used the Seliwanoff[5] resorcin test.

We had hoped that by careful determination of the sugar threshold we might receive some aid in the quantitative estimation of the extent of the particular functional disturbance we were studying, but such a procedure unhappily was impossible, because of the practically complete absence of any other quantitative standard of functional deficiency. A clinically mild hyperthyroidism, for instance, might show a markedly reduced sugar tolerance, whereas some of the severest cases gave only a slightly lowered tolerance.

As might be *a priori* expected, the interpretation of results in a scientific manner has been exceedingly difficult. Undoubtedly, the greatest trouble arises from the present state of obscurity surrounding the functions of the various glands of internal secretion. If in a case of suspected hyperpituitarism we could assume that a positive tolerance test meant hyperactivity of the hypophysis, we might prove almost anything by our results, but such a deduction is clearly arguing in a circle and only obscures the further elucidation of a difficult problem. If, on the other hand, we had incontrovertible proof that the suspected condition actually existed, then we would be safe in interpreting our result, according to the suspicion. But unfortunately absolute clinical or anatomical proof in most cases is an impossibility. Therefore, it were the better part of wisdom to consider our results from an ultraconservative point of view, and not attempt to draw any sweeping conclusions, even when the experience of a certain series would seem to point a given way. In many of our cases there can be no doubt that the test has been of distinct individual diagnostic benefit, just as a leukopenia may be of distinct benefit in suggesting *Bacillus typhosus* as the cause of an obscure fever; but we must insist on the distinction between a specific group reaction, and an occasional individual reaction in that group.

Probably the most logical method of presenting our results is by analyzing the various "group reactions." It is impossible in such a large series to go into detail in the different cases, tempting as such a pursuit might be.

The table is merely a summary of the work and includes only those cases in which the diagnosis was apparently certain. The positive dextrose tests are based on a dosage of 100 or 125 gm., while most of the levulose tests were made with 90 gm. The larger percentage of "positives" with levulose in almost all the group headings indicates clearly the well-known lower tolerance of the human organism for levulose.

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[5] Strouse and Friedman: Levulosuria, with a Report of an Unusual Case, The Archives Int. Med., 1912, ix, 99.

The forty-eight tests on miscellaneous liver conditions add nothing new to what is already known of the tests in so-called "hepatic insufficiency," and show clearly enough that a conception of the term can not be defined on a basis of carbohydrate tolerance. The apparently sharp differentiation in the results of the tests in thyroid disturbance is much more striking, and is probably due to the more definite understanding of the clinical states of hyper- and hypoactivity of this gland. Possibly we should have been more specific in the classification of our cases of hypophyseal disease, but we do not feel that the present state of knowledge warrants a sharp line between hyper- and hypo-activity in individual cases, and the attempt to draw the line in carbohydrate work would only obscure the problem. The pluriglandular syndromes are interesting and perhaps significant in that they are the only group in the list showing more positive dextrose than levulose tests. As for the "neurasthenia" group, who can tell the mystery into which we grope? Probably many of the neurasthenias of to-day will be functional gland disturbances of the future, but it would be misguided science and erroneous logic to use carbohydrate tolerance as at present understood as a means of classification. The miscellaneous group includes all sorts

Group Reactions

Group	Levulose		Dextrose		Total
	Number	Per cent. Positive	Number	Per cent. Positive	
Liver—					
Cirrhosis	8	12.5	10	70	18
Cholecystitis Pancreatitis	6	66.6	2	100	8
Carcinoma liver and pancreas.	2	100	2	100	4
Catarrhal jaundice	4	50	4	50	8
Thyroid—					
Simple goiter	5	0	3	0	8
Hyperthyroidism	18	50	11	63.5	29
Hypothyroidism.	3	0	..	..	3
Hypophysis	7	43	3	67	10
Pluriglandular	7	58	5	20	12
Neurasthenia	33	12	14	28	47
Miscellaneous	30	10	17	35	47

of diseases seen in a hospital for acute diseases, and the same commentary might be made here as was just made on the neurasthenia group.

CONCLUSION

From this clinical study we feel justified in stating that whereas tests for carbohydrate tolerance, when grouped as we have grouped our results, may indicate in a general way functional disability of an organ under suspicion, the tests can never be interpreted in an absolutely specific manner. The possibility of the implication of other organs must always be borne in mind. Functional inefficiency of one gland sufficient to lower the threshold of carbohydrate metabolism may be entirely compensated for by functional hyperactivity of a correlated gland. Even in apparently clean-cut individual cases the tests do not follow the course one might logically expect, and therefore in doubtful cases their value must remain very limited.

It gives us extreme pleasure to express our gratitude to the attending and house staffs of The Michael Reese Hospital for courtesies and assistance extended to us in the course of this study.

29 East Madison Street.—104 South Michigan Avenue.

THE POTASSIUM CONTENT OF CEREBROSPINAL FLUID IN VARIOUS DISEASES *

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It is now well recognized that certain inorganic ions play a most important part in the proper functioning of cells. Through the researches of Macallum[1] and Macdonald[2] it is known that potassium is present in the nerve-fibers and not in the nerve-cells, and that it was not until some change had taken place in the axis cylinder that potassium was liberated in an ionic state. It might be true, therefore, on the basis of these observations that in any disease with changes in the axis cylinders we might expect to find that the cerebrospinal fluid contained an increased amount of potassium.

Rosenheim[3] asserted that in all cases of acute degenerative insanity in which the presence of cholin was demonstrated in the cerebrospinal fluid, potassium in relatively large amounts could, also, be found; but in the cases in which cholin was absent, very little potassium was found. Myers[4] has shown, however, that the increased potassium content is better explained on the basis of post-mortem changes in the fluid rather than the degenerative changes found in dementia paralytica, and, also, that results obtained with post-mortem fluid cannot be compared with fluid obtained during life. He found little variation in the amount of potassium during life, but after death the potassium content was always increased about four times. Salkowski thinks that the potassium is high in acute cases and low in chronic cases.

In the literature one finds that almost all of the estimations of potassium in cerebrospinal fluid have been carried out on the centrifugated fluid; and as potassium is found in considerable amounts in the blood-cells, it seemed to us better to use fluid that was not centrifuged for the estimations. This is important, also, on account of the rapid cytolysis of the formed elements of this fluid. We also carried out the estimations soon after the fluid was removed, and on account of the rapid post-mortem increase in the potassium content, the fluid in all cases was obtained during the life of the patient.

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* Submitted for publication, May 19, 1914.

* From the Biochemical and Pathological Laboratories of the Western Pennsylvania Hospital.

[1] Macallum: Jour. Physiol., 1905, xxxii, 125.

[2] Macdonald: Proc. Roy. Soc., 1905, lxxvi, B, 329. Macdonald and Finch: Jour. Physiol., 1907, xxxv, 38.

[3] Rosenheim: Jour. Physiol., 1907, xxxv, 465.

[4] Myers: Jour. Biol. Chem., 1909, vi, 115.

Ziveri[5] reports that he has found potassium constantly in the cerebrospinal fluid from fifteen epileptics. As it is constantly present in normal fluid, however, it is difficult to see what inference could be drawn from this observation.

The potassium was estimated by the sodium cobalt-nitrite method of Drushel[6] as modified by Myers. In Tables 1, 2 and 3 we have summarized all the potassium estimations in cerebrospinal fluid that have been published.

TABLE 1.—List of Estimations from the Literature

Investigator	K_2O in 10 c.c. Fluid mg.	Source of Fluid
C. Schmidt[1]	14.3	Acute hydrocephalus
C. Schmidt[1]	10.4	Acute hydrocephalus
Coriat[2]	8.3	Hydrocephalus
Hilger[3]	6.9	Chronic hydrocephalus
Salkowski[4]	3.6	Chronic hydrocephalus
Panzer[5]	3.0	Hydrocephalus
C. Schmidt[1]	2.7	Chronic hydrocephalus
Nawratski[6]	2.4	Calf
Nawratski[6]	1.9	Calf
Yvon[7]	2.1	Chronic hydrocephalus
Halliburton[8]	1.8	Chronic hydrocephalus
Zdarek[9]	1.7	Meningocele
Mestrezat[10]	3.7	Hydrocephalus
Mestrezat[11]	2.5	Mixture of twenty normal cerebrospinal fluids
Stscherbakoff	10.8	Spina bifida

1. Schmidt: Physiol. Chem. (Hoppe-Seyler's), 1881, 604.
2. Coriat: Am. Jour. Physiol., 1903, x, 111.
3. Hilger: Centralbl. f. d. med. Wissensch., 1867, No. 56.
4. Salkowski: Festschr. f. M. Jaffe, 1901, p. 263.
5. Panzer: Wien. klin. Wchnschr., 1899, xii, 805.
6. Nawratski: Ztschr. f. physiol. Chem., 1897, xxiii, 532; Arch. f. Anat. u. Physiol., 1897, p. 156.
7. Yvon: Jour. de pharm. et chim., 1877, Series 4, xxvi, 240.
8. Halliburton: Jour. Physiol., 1889, x, 232.
9. Zdarek: Ztschr. f. physiol. Chem., 1902, xxxv, 201.
10. Mestrezat: Bull. Soc. chim., 1911, ix, 683.
11. Mestrezat: Jour. de pharm. et chim., 1909, xxix, 472.

Table 4 contains the results obtained in this study as to the potassium content of *uncentrifuged* cerebrospinal fluid obtained *during life.* We also thought it would be of interest to compare the amount of potassium found with the globulin reaction and with the cell-count.

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[5] Ziveri: Zentralbl. f. Biochem., 1909, viii, 198.

[6] Drushel: Am. Jour. Sc., 1907, xxiv, 433; ibid., 1908, xxvi, 555; Ztschr. f. anorgan. Chem., 1907, lvi, 223; 1909, lxi, 137.

TABLE 2.—Potassium Content of Cerebrospinal Fluid Obtained after Death*

Diagnosis	K₂O in 10 c.c. Fluid mg.
Dementia paralytica	11.9
Dementia paralytica	6.9
Senile dementia	7.8
Imbecility	9.3
Dementia praecox	8.6
Dementia paralytica	8.4
Dementia paralytica	11.9
Dementia paralytica	12.0
Dementia paralytica	9.7
Dementia paralytica	8.3
Epilepsy	7.0
Alcoholic delusional insanity	12.7
Alcoholic delusional insanity	10.3
Senile dementia	9.9
Senile dementia	10.6
Senile dementia	12.2
Senile dementia	8.7
Organic dementia	7.2
Melancholia	10.7
Maniac depressive insanity	10.7
Dementia praecox	7.0
Dementia praecox	9.7

* Myers: Jour. Biol. Chem., 1909, vi, 115.

TABLE 3.—Potassium Content of Cerebrospinal Fluid Obtained During Life*

Diagnosis	K₂O in 10 c.c. Fluid mg.
Dementia paralytica	2.2
Dementia paralytica	2.8† 3.4
Dementia paralytica	2.1
Dementia paralytica	1.8
Dementia paralytica	2.0
Dementia paralytica	1.8† 2.2
Dementia paralytica	1.7
Dementia paralytica	3.3
Dementia paralytica	3.0
Dementia paralytica	2.3
Dementia paralytica	2.1
Epileptic insanity	1.7
Epileptic insanity	2.2
Alcoholic pseudoparesis	2.1
Korsakoff's psychosis	2.5

* Myers: Jour. Biol. Chem., 1909, vi, 115.
† Estimations made on different days.

TABLE 4.—LIST OF ESTIMATIONS CARRIED OUT BY THE AUTHORS

Diagnosis	K_2O in 10 c.c. Fluid mg.	Cell-Count		Globulin Test
		Leukocytes	Erythrocytes	
Normal	6.1	2	0	—
Normal	5.2	1	0	—
Normal	3.6	1	Few	—
Dementia praecox	4.4	67	Occasional	+
Dementia praecox	4.6	2.5	0	—
Dementia praecox	3.4	129	572	+
Dementia praecox	5.0	1	......	—
Dementia praecox	2.2	17	......	—
General paresis	1.7	28.5	......	+
General paresis	3.0	20	0	+
General paresis	6.2	6.5	0	+
Cerebrospinal lues	9.1	9.5	0	+
Cerebrospinal lues	2.4	1	0	+
Cerebrospinal lues	7.3	7.5	......	+
Cerebrospinal lues	4.1	56	......	+
Cerebrospinal lues	3.8	5	......	—
Cerebrospinal lues	8.1	13	0	—
Cerebrospinal lues	5.4	31	Several	+
Cerebrospinal lues	5.2	58	0	+
Chronic alcoholism	1.8	1.5	0	—
Acute alcoholism	8.6	1	0	—
Acute alcoholism	3.2	3	0	—
Presenile dementia	8.6	1	0	—
Encephalitis	3.3	2	0	+
Tuberculous meningitis...	5.3	214	0	+
Tuberculous meningitis...	5.7	42	0	+
Meningitis	18.3	648	0	∓
Neurasthenia	2.5	2	0	—
Neurasthenia	5.2	1.5	0	—
Tertiary lues	2.0	34	0	+
Typhoid fever	3.8	3	0	—

CONCLUSIONS

1. The potassium content of uncentrifuged cerebrospinal fluid is higher than that of the centrifuged fluid.

2. No relation was found between the number of cells in the fluid and its potassium content.

3. No relation was found between the potassium content and the globulin reaction.

4. We do not think that it is possible on the basis of the potassium content to draw any conclusions as regards the character of the condition present in the cerebrospinal system.

5. The potassium content of the cerebrospinal fluid in thirty-one cases is presented. One of these cases with a very high potassium

content (18.3 mg.) was extremely acute, and this one case seems to bear out Salkowski's assertion that the potassium content is high in the acute cases.

6. The potassium content of the cerebrospinal fluid is not increased in degenerative diseases of the cerebrospinal system.

5737 Forbes Street.—1130 Heberton Street.

THE RELATION BETWEEN THE PHENOLSULPHONEPHTHALEIN EXCRETION IN THE URINE AND THE NON-PROTEIN NITROGEN CONTENT OF THE BLOOD IN HUMAN CASES *

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AND
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BOSTON

Last year one of us[1] in conjunction with Drs. Fitz, Folin and Denis compared the phenolsulphonephthalein excretion test in the urine with the non-protein nitrogen content of the blood in rabbits, in which an acute nephritis had been produced by the injection of uranium nitrate. It was found in this study that, although these two tests for renal function in a general way responded similarly in acute experimental nephritis in rabbits, there were certain variations. The excretion of the dye in the urine seemed to be a very fair indication of the rabbit's renal ability at the time of injection. On the other hand, the amount of retained non-protein nitrogen in the blood could be used as an indication of the renal efficiency at the moment only, provided the previous condition of the kidney was considered. No conclusions were drawn with regard to the prognostic value of these two tests in this study.

It was decided to continue during the past winter the comparative study of these two tests for renal function in a variety of human cases, especially those in which nephritis existed. It must be remembered that in the human cases more than one organic lesion is frequently present which may complicate the interpretation of the results from these two tests. It was hoped that the relative prognostic value of these tests would also be brought out by this study. In addition the systolic and diastolic blood-pressure and the pulse-pressure were studied in order to see if there was any relation between the height of the blood or pulse-pressure and the other tests.

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* Submitted for publication, June 1, 1914.

* From the Medical Clinic of the Peter Bent Brigham Hospital and the Department of Medicine, Harvard University.

[1] Frothingham, C., Fitz, R., Folin, Otto, and Denis, W.: The Relation Between Non-Protein Nitrogen Retention and Phenolsulphonephthalein Excretion in Experimental Uranium Nephritis, The Archives Int. Med., 1913, xii, 245.

With regard to these two and other tests for renal function much has been written. It is not our intention to attempt to review the literature on the subject. Fitz[2] has taken up the subject of tests for renal function in a recent communication in which he gives about forty references to recent articles. From these the earlier works on the subject may readily be found.

It must be borne in mind that no single test as yet devised indicates the entire renal function. The phenolsulphonephthalein excretion test and the non-protein nitrogen content of the blood are in favor, at present, because they seem to indicate the renal function as a whole better than any other single test. The latter test has been rendered practical by the simple and accurate method for determination of the non-protein nitrogen in the blood introduced by Folin and Denis.[3] Recently Agnew[4] has reported on a comparative study of these two tests. He concludes that when the excretion of phenolsulphonephthalein is below 40 per cent. there is a definite increase in the incoagulable nitrogen of the blood except in a case of pyelonephritis and in cases with broken cardiac compensation. He places his standard for normal non-protein nitrogen in the blood at 40 to 50 mg. per hundred c.c., which is higher than that set by Folin and Denis.[5] Our study makes us feel that the standard for the non-protein nitrogen content set by Folin and Denis at between 20 and 30 mg. per hundred c.c. of blood is the proper one for the normal. Folin, Denis and Seymour[6] have also compared these two tests and the blood-pressure. They find little if any relation between the height of the blood-pressure and the nitrogen retention.

The cases reported below were practically all in the wards of the Peter Bent Brigham Hospital. The phenolsulphonephthalein tests were made by one of us or by the house officers. Sufficient comparisons were made to see that the house-officer's readings and ours were practically the same. For this test 6 mg. of phenolsulphonephthalein were injected intramuscularly as originally described by Rowntree and Geraghty.[7] The urine was collected at the end of one hour and again at the end of the second hour. The readings were made independently on the two specimens, and the sum of the two recorded. Nothing of importance could be noted in recording separately the results for each hour. The phenolsulphonephthalein test was as a rule made on the same day, occasionally on the next day to that on which the blood was collected for the determination of the non-protein nitrogen.

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[2] Fitz: Amer. Jour. Med. Sc., in press, 1914.

[3] Folin and Denis: Jour. Biol. Chem., 1912, xi, 493.

[4] Agnew, J. H.: Comparative Study of Phenolsulphonephthalein Elimination and the Incoagulable Nitrogen of the Blood in Cardiorenal Diseases, The Archives Int. Med., 1914, xiii, 485.

[5] Folin and Denis: Jour. Biol. Chem., 1913, xiv, 29.

[6] Folin, D., Denis, W., and Seymour, M.: The Non-Protein Nitrogenous Constituents of the Blood in Chronic Vascular Nephritis (Arteriosclerosis) as Influenced by the Level of Protein Metabolism, The Archives Int. Med., 1914, xiii, 224.

[7] Rowntree and Geraghty: Jour. Pharmacol. and Exper. Therap., 1910, i, 579.

For the estimation of the non-protein nitrogen, blood was taken from the vein and mixed with potassium oxalate crystals to prevent clotting. Five c.c. of this blood were mixed with acetone-free methyl alcohol up to 50 c.c. and allowed to stand for at least two hours. The non-protein nitrogen was then estimated by the method as originally described by Folin and Denis. In most of the cases the nitrogen determinations were made independently by both of us. Usually the results agreed within a few milligrams. The higher reading is recorded in the table, as experience taught us that it was easier to lose some of the nitrogen in the test than to overestimate it. In a few cases in which the results did not agree within reasonable limits the observation was not recorded.

In this work 140 observations were made on 77 individuals. Most of the cases were diagnosed as chronic nephritis with or without other organic lesions. Several cases, however, in which there was no apparent renal disease were included.

The results are recorded in the accompanying table. In the column designated "disease" are recorded the more important diagnoses made at the hospital, and a statement with regard to the presence of edema and cardiac compensation at the time of the test.

It will be readily seen from this table that there are many cases diagnosed clinically and by laboratory tests as those of chronic nephritis, which show a normal phenolsulphonephthalein elimination and a normal non-protein nitrogen content in the blood.

In order to bring out the relation between the two tests and the blood-pressure more clearly the accompanying chart has been prepared. The nitrogen determinations have been divided into groups according to the amount per hundred c.c. of blood thus:

Group 1 includes 13 non-protein nitrogen determinations between 10- 20
Group 2 includes 52 non-protein nitrogen determinations between 20- 30
Group 3 includes 32 non-protein nitrogen determinations between 30- 40
Group 4 includes 12 non-protein nitrogen determinations between 40- 50
Group 5 includes 3 non-protein nitrogen determinations between 50- 60
Group 6 includes 2 non-protein nitrogen determinations between 60- 70
Group 7 includes 7 non-protein nitrogen determinations between 70- 80
Group 8 includes 3 non-protein nitrogen determinations between 80- 90
Group 9 includes 4 non-protein nitrogen determinations between 90-100
Group 10 includes 4 non-protein nitrogen determinations between 100-200
Group 11 includes 6 non-protein nitrogen determinations between 200-300
Group 12 includes 2 non-protein nitrogen determinations between 300-400

RECORDS OF NON-PROTEIN NITROGEN IN BLOOD AND PHENOLPHTHALEIN EXCRETION

GROUP 1

Hosp. No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
396	13.0	57	115- 75	Diabetes mellitus.
454	14.7	78	115- 64	Diabetes mellitus.
577	16.1	47	140- 90	Chronic nephritis, no edema.
536	16.6	50	110- 65	Pulmonary and peritoneal T. B., edema.
591	17.4	43	130- 90	Myxedema, no edema.
728	18.5	56	130- 90	Diabetes, myocarditis, no edema.
604	19.3	72	130- 90	Chlorosis, no edema.
913	19.4	60	105- 70	Diabetes insipidus, no edema.
680	19.5	41	190-100	Chronic nephritis, diabetes, edema.
650	19.5	75	170-100	Chronic nephritis, hypertension, slight edema.
818	19.5	59	130- 90	Pleurisy serofibrinous, no edema.
603	19.6	75	194-112	Hyperthyroidism, no edema.
836	19.6	38	230-130	Chronic nephritis, hypertension, no edema.

GROUP 2

Hosp. No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
513	20.7	40	120- 85	Chronic cardiac valvular and fibrillation, broken compensation, edema.
589	20.8	37	198-115	Chronic nephritis, chronic myocarditis, arteriosclerosis, edema and broken compensation.
590	20.8	29	135- 90	Chronic nephritis, chronic cardiac, no edema, compensation.
680	20.9	30	190- 90	Chronic nephritis, diabetes.
939	20.9	25	115- 80	Chronic cardiac, myxedema, broken compensation.
624	21.1	55	210-130	Chronic nephritis, arteriosclerosis, hypertension, no edema, compensation.
684	21.2	47	220-130	Chronic nephritis, hypertension, compensation, no edema.
428	21.5	51	122- 80	Chronic nephritis, slight edema.
675	21.6	55	150- 90	Chronic nephritis, no edema, compensation.
631	21.7	37	175-110	Chronic nephritis, chronic cardiac, slight edema, broken compensation.
942	21.8	48	170- 85	Diabetes mellitus.
G	21.9	50	....	Chronic nephritis, no edema, compensation.
739	22.8	58	235- 95	Chronic nephritis, hypertension, compensation, no edema.
672	23.0	42	200-120	Chronic nephritis, arteriosclerosis, no edema.
828	23.1	61	130- 85	Chronic cardiac, broken compensation, no edema.
897	23.2	59	200- 95	Pelvic cancer, no edema, compensation.
548	23.2	35	210- 80	Chronic nephritis and endocarditis, no edema
460	23.3	50	180-120	Specific aortitis, chronic myocarditis, no edema.
684	23.3	70	210-110	Chronic nephritis, hypertension, compensation, no edema.
1030	23.4	75	230-170	Cirrhosis liver, hypertension, compensation, no edema.
684	23.8	69	220-110	Chronic nephritis, hypertension, no edema, compensation.
1075	23.8	55	192-110	Chronic nephritis, edema, compensation.
428	23.9	45	145-100	Chronic nephritis, no edema.

Records of Non-Protein Nitrogen in Blood—(Continued)

Group 2.—(Continued)

Hosp. No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
513	24.2	40	120- 70	Chronic cardiac, valvular, fibrillating, slight edema, broken compensation.
1046	24.7	40	170-110	Chronic nephritis, hypertension, no edema, compensation.
428	24.7	52	....	Chronic nephritis, slight edema.
620	24.9	20	180-104	Chronic valvular disease, edema.
986	25.3	50	190-125	Chronic nephritis, hypertension, moderate edema, fair compensation.
624	25.4	45	225-124	Arteriosclerosis, hypertension, no edema, fair compensation.
722	25.5	46	210-150	Arteriosclerosis cerebral, no edema.
739	25.5	52	215- 80	Chronic nephritis, hypertension, compensation, no edema.
571	25.6	60	220-150	Chronic nephritis, syphilis, no edema.
816	25.9	51	190-110	Chronic nephritis, cirrhosis of liver with edema, hypertension.
657	26.1	65	145-100	Chronic nephritis, no edema, tonsillitis.
316	26.1	67	135- 95	Chronic nephritis, cirrhosis, arteriosclerosis.
963	26.1	43	165- 95	Chronic arthritis, no edema, compensation.
1023	26.3	40	215-150	Chronic nephritis, hypertension, slight edema, compensation.
684	27.0	58	185-110	Chronic nephritis, hypertension, no edema, compensation.
920	27.0	58	120- 50	Chronic cardiac, no edema, compensation.
684	27.7	57	210-120	Chronic nephritis, hypertension, no edema, compensation.
876	27.7	50	130- 70	Chronic cardiac, broken compensation.
991	28.0	70	130- 80	Diabetes, no edema, compensation.
782	28.4	66	160- 85	Chronic nephritis, rheumatism, hypertension, compensation, no edema.
1029	28.5	66	185- 70	Chronic nephritis, hypertension, no edema, compensation.
624	28.5	50	210-130	Chronic nephritis, arteriosclerosis, compensation, no edema.
928	28.8	30	170-100	Chronic nephritis, myocarditis, hypertension, no edema.
589	29.0	50	195-110	Chronic nephritis, myocarditis, arteriosclerosis, slight edema, fair compensation.
675	29.0	43	150- 90	Chronic nephritis, no edema, compensation.
590	29.2	33	145-110	Chronic nephritis, chronic cardiac, no edema, fair compensation.
672	29.4	48	180-120	Chronic nephritis, arteriosclerosis, no edema.
609	29.8	41	218-120	Chronic nephritis, hypertension, arteriosclerosis, no edema, compensation.
672	29.9	43	220-140	Chronic nephritis, arteriosclerosis, no edema.

Group 3

Hosp. No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
817	30.0	53	220-130	Chronic nephritis, hypertension, no edema, compensation.
836	30.3	46	210-120	Chronic nephritis, hypertension, no edema.
701	31.0	45	160-100	Chronic nephritis, chronic myocarditis, no edema, fair compensation.
837	31.0	49	115- 72	Chronic cardiac, broken compensation, no edema.

RECORDS OF NON-PROTEIN NITROGEN IN BLOOD—(Continued)

GROUP 3.—(Continued)

Hosp No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
856	31.2	26	100- 80	Chronic nephritis, chronic myocarditis, edema, broken compensation.
876	31.2	30	240-160	Chronic nephritis, hypertension, no edema, compensation.
624	31.2	50	210-120	Chronic nephritis, arteriosclerosis, compensation, no edema.
719	31.4	28	128- 80	Chronic cardiac, fair compensation.
1045	31.6	50	220-120	Chronic nephritis, hypertension, no edema compensation.
817	31.8	63	220-145	Chronic nephritis, hypertension, no edema, compensation.
678	31.9	30	165-130	Chronic nephritis, no edema, compensation, hypertension.
609	32.8	47	225-128	Chronic nephritis, hypertension, arteriosclerosis, no edema, compensation.
782	33.3	52	175-115	Chronic nephritis, rheumatism, hypertension, compensation, no edema.
763	33.3	52	200-120	Chronic nephritis, arteriosclerosis, hypertension, slight edema, fair compensation.
791	33.4	75	210-150	Chronic nephritis, arteriosclerosis, chronic myocarditis, hypertension, no edema, compensation.
873	33.5	31	160- 65	Chronic nephritis, no edema, compensation.
675	34.0	54	170-105	Chronic nephritis, slight edema, compensation.
873	34.2	62	135- 60	Chronic nephritis, no edema, compensation.
631	34.7	49	190-120	Chronic nephritis, chronic cardiac, no edema, fair compensation.
854	34.9	42	130- 90	Gastroptosis, temporary hypertension.
817	35.2	51	195-130	Chronic nephritis, hypertension, no edema, compensation.
678	35.4	33	164-	Chronic nephritis, no edema, compensation, hypertension.
918	35.4	40	....	Chronic nephritis, compensation, no edema.
848	35.7	48	145- 90	Chronic cardiac, acute rheumatism, broken compensation, no edema.
684	35.9	65	210-110	Chronic nephritis, hypertension, no edema, compensation.
739	36.5	32	230-110	Chronic nephritis, hypertension, compensation, no edema.
343	37.1	43	....	Chronic cardiac, arteriosclerosis.
739	37.5	35	200- 90	Chronic nephritis, hypertension, compensation, no edema.
763	39.0	46	205-130	Chronic nephritis, arteriosclerosis, hypertension, no edema, fair compensation.
763	39.0	48	200-120	Chronic nephritis, arteriosclerosis, hypertension, edema.
428	39.1	45	140- 90	Chronic nephritis, edema free.
762	39.8	18	175-125	Chronic nephritis, hypertension, no edema, compensation.

GROUP 4

589	43.4	22	170-100	Chronic nephritis, chronic myocarditis, arteriosclerosis, no edema, fair compensation.
762	43.4	35	195-125	Chronic nephritis, hypertension, edema, broken compensation.

RECORDS OF NON-PROTEIN NITROGEN IN BLOOD—(Continued)

GROUP 4.—(Continued)

Hosp. No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
762	44.7	16	180-130	Chronic nephritis, hypertension, no edema, fair compensation.
631	45.7	25	210-142	Chronic nephritis, chronic cardiac, broken compensation, edema.
627	46.9	17	155- 90	Chronic nephritis, arteriosclerosis, edema, broken compensation.
678	46.9	42	160-110	Chronic nephritis, no edema, compensation, hypertension.
762	47.6	25	190-120	Chronic nephritis, hypertension, slight edema, fair compensation.
1127	47.8	35	238-135	Chronic nephritis, diabetes, hypertension, pending uremia, compensation.
631	47.8	37	175-135	Chronic nephritis, chronic cardiac broken compensation, edema.
974	48.1	25	190-120	Chronic nephritis, hypertension, no edema, compensation.
675	49.0	37+	140- 85	Chronic nephritis, no edema, compensation.
739	49.0	23	220- 90	Chronic nephritis, hypertension, compensation, no edema.

GROUP 5

Hosp. No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
762	50.0	36	175-110	Chronic nephritis, hypertension, slight edema, fair compensation.
974	54.1	25	180-120	Chronic nephritis, hypertension, no edema, compensation.
762	56.1	18	195-130	Chronic nephritis, hypertension, no edema, fair compensation.

GROUP 6

Hosp. No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
627	62.8	34	140- 90	Chronic nephritis, chronic cardiac, slight edema, broken compensation.
950	69.9	48	140- 90	Chronic nephritis, chronic cardiac, slight edema, broken compensation.

GROUP 7

Hosp. No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
918	70.9	14	240-170	Chronic nephritis, hypertension, edema, broken compensation.
950	73.0	27	140- 90	Chronic nephritis, chronic cardiac, edema, broken compensation.
918	75.7	25	260-190	Chronic nephritis, hypertension, no edema, fair compensation.
762	76.8	15	170-120	Chronic nephritis, hypertension, no edema, compensation.
762	78.7	12	150-110	Chronic nephritis, hypertension, no edema, compensation.
1056	78.8	Trace	210-115	Chronic nephritis, hypertension, pending uremia, slight edema, fair compensation.
918	79.6	20	268-198	Chronic nephritis, hypertension, slight edema, fair compensation.

RECORDS OF NON-PROTEIN NITROGEN IN BLOOD—(Continued)

GROUP 8

Hosp. No.	Blood Nitrogen mg. per 100 c.c.	Phthalein Total 2 Hrs.	Blood-Pressure	Disease
1056	87.0	Trace	210-105	Chronic nephritis, pending uremia, hypertension, compensation, no edema.
762	88.0	33	140-100	Chronic nephritis, hypertension, no edema, compensation.
655	89.2	3	220-145	Chronic nephritis, no edema, pending uremia.
GROUP 9				
621	90.0	3	280+-160	Chronic nephritis, pending uremia, hypertension, no edema, compensation.
573	90.4	3	240-130	Chronic nephritis, no edema.
655	96.1	28	230-144	Chronic nephritis, no edema, pending uremia.
655	96.1	Trace	230-144	Chronic nephritis, no edema, pending uremia.
GROUP 10				
895	117.0	0	194-120	Chronic nephritis, uremia, no edema, compensation.
573	117.0	2	230-130	Chronic nephritis, no edema, pending uremia.
655	129.8	0	220-170	Chronic nephritis, no edema, pending uremia.
1056	129.8	Trace	210-100	Chronic nephritis, uremia, hypertension, no edema, compensation.
GROUP 11				
573	200.0	0	215-130	Chronic nephritis, uremia, no edema.
673	209.0	0	195-124	Chronic nephritis, hypertension, no edema.
1058	212.0	Trace	....	Chronic nephritis, uremia, edema, broken compensation.
655	278.0	0	230-165	Chronic nephritis, uremia, no edema.
673	285.7	0	205-130	Chronic nephritis, hypertension, uremia, no edema.
673	288.0	0	195-110	Chronic nephritis, hypertension, pending uremia, no edema.
GROUP 12				
655	330.0	Anuric	180-120	Chronic nephritis, uremia, no edema.
655	347.0	Anuric	180-110	Chronic nephritis, uremia, no edema.

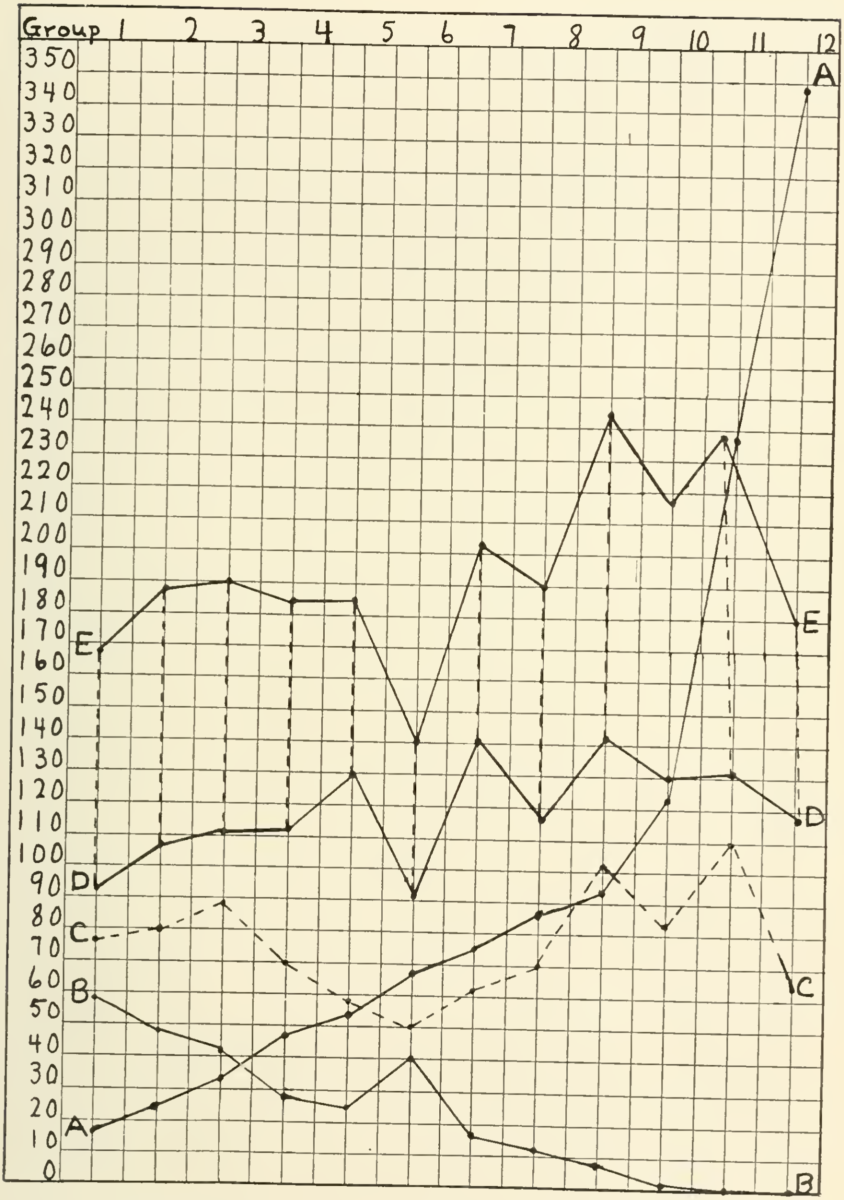

Chart showing as follows: .—A-A, Non-protein nitrogen in the blood; B-B, phenolsulphonephthalein excretion; C-C, pulse-pressure; D-D, diastolic blood-pressure; E-E, systolic blood-pressure.

The average of the non-protein nitrogen determinations in each group has been taken and also the average of the phenolsulphonephthalein excretion for these same cases. In averaging the blood-pressures it seems fairer to leave out the cases in which chronic nephritis was not present because the blood-pressure in these cases was considerably lower. The non-nephritic cases did not affect the non-protein nitrogen or phenolsulphonephthalein averages, for they occurred only in those groups in which the non-protein nitrogen and phenolsulphonephthalein fell within normal limits.

From the accompanying chart it is readily seen that the phenolsulphonephthalein excretion steadily falls as the amount of non-protein nitrogen in the blood increases, except in Group 6, which is composed of only two cases. When the non-protein nitrogen reaches the hundred mark the elimination of the dye within the two hours practically ceases. Marked increase in non-protein nitrogen in the blood of over 150 mg. per hundred c.c. appeared from our cases to come on just a few days preceding death, and we looked on it as a terminal phase of the disease. The relation between the blood-pressure and the pulse-pressure and the other tests is not so definite. The systolic pressure averaged slightly higher in the groups with the nitrogen above 70 mg. (except in Group 12) than in Groups from 1 to 6. The pulse-pressure, however, showed no definite relation to the other tests.

If, on the other hand, the observations are considered individually, it is found that considerable variation occurs between the phenolsulphonephthalein excretion in different cases in the same group in which of course the non-protein nitrogen content varies but little. The same may also be said of the blood-pressure. Thus, for example, in Group 1, the phenolsulphonephthalein excretion varies from 38 per cent. to 78 per cent., in Group 2 from 20 per cent. to 75 per cent., in Group 3 from 18 per cent. to 75 per cent. In the other groups, except 10, 11 and 12, variations occur of anywhere from 15 per cent. to 35 per cent. in a group.

In order to discover if there was any complicating factor which might influence the excretion of the phenolsulphonephthalein, the clinical records were carefully studied in those cases in which the individual phthalein excretion was 10 per cent. below or above the average of its group. No pathological condition was found constantly present to account for the increase in phenolsulphonephthalein excretion. Several reports have appeared in the literature from time to time in which the phenolsulphonephthalein excretion has been unexpectedly high without any satisfactory explanation being given, and we feel that our work has not thrown any light on that point.

In a considerable number of the cases in which the phenolsulphonephthalein excretion was below the average either edema or broken compensation was shown to some degree. We therefore agree with the view already expressed by others that the excretion of the phenolsulphonephthalein is influenced by passive congestion but that the non-protein nitrogen content in the blood is not. On the other hand, patients with broken cardiac compensation frequently excreted a normal amount of phthalein. In a moderate number of cases, however, a surprisingly low phenolsulphonephthalein excretion was shown for which no cause could be found either from the clinical findings or laboratory tests. It seems fair, therefore, to say that a considerable individual variation above or below the expected average may occur in the phenolsulphonephthalein test for which no cause can be found. The relation between the non-protein nitrogen content of the blood and the clinical findings is fairly uniform. No non-nephritic cases showed an increase of non-protein nitrogen in the blood. All the patients with the non-protein nitrogen content above 50 mg. per hundred c.c. of blood either died or left the hospital with evidence, clinically and from other laboratory tests, of a severe nephritis. In some of these cases the phenolsulphonephthalein excretion was at times fairly good. The cases with a non-protein nitrogen content above 35 mg. per hundred c.c. of blood, except when it was forced up by special diet, usually appeared clinically to be a more severe grade of nephritis than the cases without such a high non-protein nitrogen content.

CONCLUSIONS

Many cases of chronic nephritis show normal phenolsulphonephthalein excretion and normal non-protein nitrogen content in the blood.

The content of non-protein nitrogen in the blood varies in a rough way inversely with the excretion of phenolsulphonephthalein in the urine in nephritis. The phenolsulphonephthalein excretion is influenced by cardiac decompensation and other unknown factors so that quite unexpected results occur in individual cases.

The content of non-protein nitrogen in the blood seems to be less influenced by cardiac and other complicating factors.

The amount of non-protein nitrogen in the blood seems to be of considerable prognostic value.

The height of the blood-pressure and the pulse-pressure do not seem to be of prognostic importance in nephritis.

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A POINT TO BE CONSIDERED IN THE USE OF THE ABDERHALDEN REACTION *

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Abderhalden has demonstrated that the parenteral introduction into the body of foreign protein or other material results in the appearance in the blood-serum of ferments capable of splitting these foreign substances into bodies which are less disharmonious with the organism. He has shown that these ferments are produced only by the presence in the blood of the corresponding material and that they seem to be specific; that is to say, that they are capable of splitting only the one particular variety of protein, or other substance, the injection of which resulted in their formation.

The dialysis method, by which proteolytic ferments may be detected, recommended by Abderhalden, consists essentially of two parts. First, about 0.5 gm. of sterile protein (which must be free from substances which react with ninhydrin) is placed with 1.0 to 1.5 c.c. of the sterile blood-serum under test, free from hemoglobin and blood-cells, in a sterile, tested, and standardized dialyzing thimble. This is then floated in 20 c.c. of sterile water in a sterile vessel and the whole incubated at blood temperature for sixteen hours. Secondly, 10 c.c. of the dialysate is boiled for exactly one minute with 0.2 c.c. of a 1 per cent. solution of ninhydrin. The appearance or non-appearance of a blue color within half an hour is an indication of the presence or absence of amino-acids, polypeptids or peptones (and other substances) and from this it is deduced that lysis of the protein material used in the test has or has not occurred, and hence that the blood-serum does or does not contain a proteolytic ferment for this particular protein. Control tests with the various substances used are obviously necessary.

The discovery of these facts has led to the suggestion that in this reaction we have a means of detecting destruction by injury or disease of any organ within the body; for it is claimed that some of the proteins of which each organ is composed are peculiar to that organ and under conditions of ordinary healthy life do not enter the blood-stream. Destruction of an organ within the body, however produced, leads to the absorption of the specific protein peculiar to it, and although some splitting and lysis probably occurs within the bodies of the migratory

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* From the Laboratory of the Illinois State Psychopathic Institute.

cells which must act as the medium for the transfer of this dead protein to the blood-stream, yet it may be that the substance which finally gains admission to the circulation still retains the essential specific molecular construction of the original tissue protein. This would then give rise to the production of proteolytic ferments in the blood-serum specific for the proteins peculiar to the organ which has been destroyed.

This suggestion has been seized by many workers with the greatest enthusiasm and within the two years which have elapsed since the publication of Abderhalden's work an enormous literature has been built up dealing with investigations into pathological conditions of all kinds. Especially true is this in regard to the disorders grouped under the head of psychiatry.

A study of this literature reveals the most contradictory and confusing results, a state of affairs which seems inevitable when the complexity of the series of chemical changes already indicated in the passage of the dead protein from the organ to the blood-stream is considered. When to this is added the rigid and exacting nature of the technic of the final test, the sources of possible error become enormous. To illustrate the situation, reference may be made to the findings in dementia praecox. Fauser claims to have demonstrated that the serum of these patients always contains ferments which will digest the proteins of sex glands and that other types of mental disorder do not show this phenomenon. He is supported by the observations of many others. Yet even among those who more or less conform to this view we find that Kafka,[1] for instance, reports the digestion of ovary by male serum and of testis by female serum, although he makes no comments on these results. Other observers, on the other hand, not only fail to find such digestion in all cases of dementia praecox, but do find it in other psychoses. Reference may be made, for instance, to the careful work of Simon,[2] who reports that in 12 per cent. of the cases of dementia praecox examined by him, the serum contained no proteolytic ferment for sex gland, while in 30 per cent. of the other forms of psychosis investigated the serum did possess this property.

So far has enthusiasm been carried that it is quite common to find authors suggest that all observations, whether by themselves or others, which do not conform to the views with which they start are the result of faulty technic, while others even suggest that the clinical diagnosis was incorrect. Even does one find commercial laboratories which make returns "positive or negative for dementia praecox."

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[1] Kafka: Ztschr. f. d. ges. Neurol. u. Psychiat., 1913, xviii, 348, Cases 11 and 31.

[2] Simon, Charles E.: The Abderhalden-Fauser Reaction in Mental Diseases with Special Reference to Dementia Praecox, Jour. Am. Med. Assn., 1914, lxii, 1701.

In view of the far-reaching conclusions which are being so confidently drawn and of the contradictory results which have been obtained, it seems that more methodical and careful study of the fundamental problems concerned is eminently desirable.

In the first place, it is difficult to understand why, if tissues like placenta and carcinoma give rise to the appearance in the blood-serum of protective enzymes, the normal tissue cells of the body should not do so likewise. That Abderhalden seems to admit that cell metabolism may have this effect is apparent from his statement in a recent paper[3] read before the American Medical Association, that "one must abandon the frequently accepted view that the appearance of protective enzymes or of new blood enzymes in general is associated with cell destruction." With this question in view it was decided to make a series of tests on the tissues and serums of healthy dogs and cats. This has led to certain findings which appear of considerable importance.

EXPERIMENTAL WORK

The animal was anesthetized with ether. The two jugular veins were dissected out and sterile cannulas were inserted into each end of the cut veins of either side. Blood for serum was drawn into sterile tubes, from a vein or sometimes from the carotid artery. The proximal ends of the veins were connected with a tank containing 0.9 per cent. salt solution. As soon as sufficient blood for serum had been collected, the blood was allowed to flow from the distal ends of the veins. At the same time the salt solution was allowed to enter the blood-stream. This arrangement was allowed to remain till the outcoming fluid was colorless or very slightly pink. Sometimes just before the death of the animal, ammonium oxalate was added to the salt solution. This solution was allowed to run into the body for about ten minutes and then the connections were made with a tank of distilled water so that this water was infused into the body instead of the salt solution.

After the infusion was over the organs were very free from blood. The body was opened and the following organs were removed: brain, thyroid, liver, spleen, pancreas, kidneys, suprarenals and sex glands.

Each of these organs was separately minced and boiled for ten or fifteen minutes with water containing about five drops of acetic acid per liter. The mixture was then filtered. The washing was repeated five to seven times. The tissues were then put into test-tubes which were stoppered with cotton plugs. These were then sterilized for half an hour in steam at 5 pounds' pressure.

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[3] The Present Status of the Investigation of the Protective Enzymes, read before the Section on Obstetrics at the sixty-fifth annual session of the American Medical Association, Atlantic City, N. J., June, 1914. This has not yet been published, but a copy was kindly furnished by Prof. A. J. Carlson of the University of Chicago.

Blood was also prepared as a substrate. It was washed, boiled and sterilized in exactly the same manner as the tissues.

The blood collected for serum was allowed to stand till the serum separated well from the clot. The serum was removed by decantation or centrifugation and then again centrifuged.

When blood was collected for serum without killing the animal, it was taken with a sterile needle from the saphenous vein.

The parts for the test were assembled as follows: Twenty c.c. of sterile distilled water was placed with a sterile pipet into each cotton-plugged sterile test-tube (1 inch in diameter and 8 or 10 inches long). The sterile dialyzing thimble was lifted with sterile forceps and held while the sterile substrate and serum, either or both, were introduced. The dialyzing tube was then let gently into the water in the test-tube and the latter again closed. A single complete test consisted of one test with serum alone in the dialyzing-tube, one test with substrate alone and one test with serum and substrate together. The combinations were incubated at 37 C. for sixteen hours. After the incubation, 10 c.c. of the dialysate was measured out into a clean large test-tube. To this was added 0.2 c.c. of a 1 per cent. solution of ninhydrin. This solution was boiled strongly for exactly one minute. The color was noted and recorded as negative, questionable, positive, double positive or triple positive, meaning respectively, no color, suspicion of color, just enough blue to be easily seen, an amount of blue about midway between the latter test and the next, and finally, so much blue that it was difficult to see through it against a white background. The result of the complete test was considered positive if the color of the dialysate from the tissue and serum was more intense than the combined color of the other two dialysates. It was considered questionable when the intensity of the color of this dialysate was equal to the other two. The test was negative either when there was no blue color in the tested dialysate of the tissue and serum or when the color was markedly less than the intensity of that of the other two combined. The dialysing thimbles had been tested first for permeability to albumin. They should not allow protein to pass through them. Egg-white was used as substrate and the biuret test for albumin employed in place of the ninhydrin.

The dialysing tubes were next tested for permeability to peptone and standardized in this respect. The regular test was carried out, except that 1 c.c. of 10 per cent. solution of peptone was used in place of the substrate and serum. Those tubes giving a test with ninhydrin of less than a double positive were discarded. Those tubes allowing peptone to pass through sufficiently to give a double positive result with

TABLE 1.—Results with a Strict Abderhalden Technic *

Tissue	No. of Tests	Immediately After Boiling for One Minute		After Standing for Half an Hour	
		Positive	Doubtful	Positive	Doubtful
Blood (1)	21	9	1	12	2
Brain (2)	20	4	1	8	3
Kidney	22	7	1	9	2
Liver (3)	32	5	0	7	2
Pancreas (4)	11	1	0	1	2
Spleen (5)	15	9	1	7	4
Suprarenal	5	1	0	2	0
Sex glands	6	3	0	3	1
Thyroid (6)	9	3	0	4	0

* Included with the foregoing figures are the results of tests in which incubation was prolonged to twenty-four hours. The number of tests and the results with this variation were:

Tissue	No. of Tests	Immediately After Boiling	After Standing One-Half Hour
1	2	Positive	Positive
2	1	Negative	Doubtful
3	2	Negative	Negative
4	2	Negative	Doubtful
5	1	Negative	Negative
6	2	Positive	Positive

TABLE 2.—Results with Strict Abderhalden Technic Expressed in Percentages

Tissue	Immediately After Boiling for One Minute		After Standing for Half an Hour	
	Positive	Negative	Positive	Negative
Blood	43	52	57	33
Brain	20	75	40	45
Kidney	32	65	41	50
Liver	16	84	22	72
Pancreas	9	91	9	73
Spleen	60	33	47	27
Supararenal ..	20	80	40	60
Sex Gland....	50	50	50	33
Thyroid......	33	67	44	56
Average ...	32	66	38	50

ninhydrin were marked and those giving a triple positive test were also marked. These two classes of tubes were kept separate and no complete test was made in which tubes of the two classes were used together.

The results of 141 tests with a strict Abderhalden technic are given in Tables 1 and 2.

TABLE 3.—Results with Incubation for Twenty-Four Hours and Substitution of 0.9 Per Cent. Salt Solution for Distilled Water in the Dialyser

Tissue	No. of Tests	Immediately After Boiling for One Minute		After Standing for Half an Hour	
		Positive	Doubtful	Positive	Doubtful
Blood	10	2	0	3	2
Brain	10	6	0	2	2
Kidney	9	5	0	4	1
Liver	12	5	0	4	2
Pancreas	5	2	0	1	1
Spleen	11	5	0	4	0
Suprarenal	4	2	1	2	1
Sex gland	5	4	0	2	2
Thyroid	6	3	0	2	2

TABLE 4.—Results in Table 3 Expressed in Percentages

Tissue	Immediately After Boiling for One Minute		After Standing for Half an Hour	
	Positive	Negative	Positive	Negative
Blood	20	80	30	50
Brain	60	40	20	60
Kidney	56	44	44	44
Liver	42	58	33	50
Pancreas	40	60	20	60
Spleen	45	55	36	64
Suprarenal ...	50	25	50	25
Sex gland ...	80	20	40	20
Thyroid	50	50	33	33
Average	49	48	34	45

The results given in Tables 1 and 2 prove quite conclusively that the serum of normal animals may frequently contain ferments capable of splitting the protein in a number of the tissues of the body. Similar results were obtained by Lichtenstein and Hage.[4]

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[4] Lichtenstein, S., and Hage: Nachweis von spez. Fermenten mit Hilfe des Dialysierverfahrens, München. med. Wchnschr., 1914, lxi, 915.

If the animals we used were all normal healthy animals, as they all seemed to be, there should have been a more uniform set of results. It seems that the results on any organ tissue should have been all positive or all negative. It was thought that possibly this variation might have been due to the length of time of incubation and to the character of the medium in which the dialyzing tube was floated. Since it takes time for enzymes to do their work, and it also takes time for substances to dialyze, and also since it takes a certain definite amount of protein decomposition products to give a perceptible ninhydrin reaction, the lengthening of the time of incubation to twenty-four hours might increase the number of positive results. Since the activity of the well-studied ferments of the body is much affected by the medium in which they act, it was thought important to maintain the original salt concentration of the serum throughout the time of incubation. This would be better accomplished by using a sterile physiological salt solution in place of the distilled water used in the original method. Therefore a number of tests were made modifying the original method in the ways just mentioned; that is, lengthening the time of incubation to twenty-four hours and using 0.9 per cent. salt solution in place of distilled water. The results of seventy-two tests are given in Tables 3 and 4.

TABLE 5.—Results in Tables 1 and 3 Combined, Expressed in Percentages

Tissue	No. Tests	Immediately After Boiling for One Minute			After Standing for Half an Hour		
		Positive	Doubtful	Negative	Positive	Doubtful	Negative
Blood	31	35	4	61	48	13	39
Brain	30	33	4	63	33	17	50
Kidney	31	39	3	58	42	10	48
Liver	44	23	0	77	25	9	66
Pancreas	16	19	0	81	12	19	69
Spleen	26	54	2	44	42	16	42
Suprarenal	9	33	11	56	44	12	44
Sex gland	11	64	0	36	45	28	27
Thyroid	15	40	0	60	40	13	47
Average	213	36	2	62	36	14	50

Tables 3 and 4 do not show any marked differences in results from those of Tables 1 and 2. The same lack of uniformity exists. As a whole, about the same percentages of positives and negatives occur as when the original method was used.

Since this alteration in the Abderhalden method did not seem to affect the results, we are justified in combining the figures of Tables 1, 2, 3 and 4 in Table 5.

In using the regular method, a number of different serums were used on a series of tissues which were composite mixtures of the same organs of several dogs. Likewise a number of tests were made on the tissues of one dog with the serums of several others. The results are given in Tables 6 and 7.

The uniformity of these results indicates that the irregularity of those previously reported was not due to differences in the serums. On the contrary, it was probably in the substrate, and most likely in its preparation. The preparation of the tissues had been done with the greatest care, and strictly according to the methods prescribed by Abderhalden. In but two cases out of some hundreds of tests made have the substrates alone given a positive ninhydrin reaction.

TABLE 6.—Tests on a Single Set of Composite Tissues with Various Serums

Donor of Serum	Blood	Spleen	Kidney	Liver
Bird-dog	+	—	..	—
Bulldog	+	—	—	—
Dog 5	+	—	—	—
Terrier B. and T.	+	?	—	

TABLE 7.—Tests on Tissues of Dog 5 with Various Serums

Donor of Serum	Blood	Brain	Spleen
Dog 8	—	—	+
Dog 4	—	—	+
Fox terrier ..	—	—	+

In reviewing these facts it occurred to us that there might be some substance or substances in the body tissues which is soluble in boiling water containing 5 drops of acetic acid per liter, capable of being broken down by a serum ferment into parts which would give a blue color with ninhydrin; whereas, before the cleavage, ninhydrin would not give the characteristic reaction. If these suppositions were correct, any or all tissues prepared as a substrate might not or might give a positive reaction according as the substrate were or were not washed free of this material.

An attempt was made to separate this hypothetical substance (or substances). The acid washings from the substrates, prepared in the usual manner, were filtered through the best grade of filter-paper. The filtrate was boiled down to a small volume and an equal volume of absolute alcohol was added. A precipitate was formed in every case. This precipitate was centrifuged out and washed about five times by centrifugation with equal volumes of water and alcohol. It was then put into a test-tube and set in boiling water for ten or fifteen minutes for the purpose of driving off the alcohol. The tube was then plugged with a cotton stopper and sterilized under steam at about 5 pounds' pressure for twenty minutes. Preparations were made in this manner from brain, kidney, liver, pancreas, spleen, suprarenal, testicle, thyroid and muscle. Emulsions of these preparations were used as substrates in the regular Abderhalden reaction. The results were as shown in Table 8.

TABLE 8.—Tests Made on Preparations from the Acid Washings of Organs in the Process of Preparing These Organs as Substrates

Tissue	Source of Tissue	Donor of Serum	Results		
			Ppte. and Serum	Ppte. Alone	Serum Alone
Brain	Composite	Collie	+	—	—
Brain	Composite	Collie	+	—	—
Kidney	Dog 9	Bulldog	+ + +	—	+
Kidney	Dog 9	Bulldog	+ + +	—	+
Liver	Dog 9	?	+	—	—
Liver	Dog 9	?	+	—	—
Pancreas	Dog 9	Terrier	+ + +	—	+ ?
Spleen	Composite	Sheep-dog	+	—	—
Spleen	Composite	Sheep-dog	+	—	—
Suprarenal	Composite	Collie	+	—	—
Suprarenal	Composite	Collie	+	—	—
Testis	Composite	Sheep-dog	+ +	—	—
Testis	Composite	Sheep-dog	+ +	—	—
Thyroid	Composite	Collie	+ +	—	—
Thyroid	Composite	Collie	+	—	—
Muscle	Bull dog	Collie	+ +	—	—
Muscle	Bull dog	Collie	+ +	—	—

Having found that blood treated like other tissue as a substrate gave some positive results, it seemed admissible to prepare blood free from hemoglobin for a further test. The blood was drawn into a flask containing ammonium oxalate. The corpuscles were centrifuged out and were then broken by being allowed to stand in distilled water over night. The mixture was then centrifuged and the residue washed till free from hemoglobin with distilled water. The solid material was placed in a test-tube plugged with cotton and then sterilized as the other substrates. An emulsion of this substance was made in sterile water and used for the Abderhalden reaction. The results were as follows:

1. Preparation from blood of dog and serum of collie dog	++
2. Preparation from blood of dog and serum of collie dog	++
1. Preparation from blood of dog alone....................	—
2. Preparation from blood of dog alone....................	—
1. Serum of collie dog alone..............................	+
2. Serum of collie dog alone..............................	+

These results show conclusively that there is in each of the tissues with which we worked a substance or a number of substances, which are washed out with acidified water, which do not give a blue color when boiled with ninhydrin, but which are, by normal serum ferments, broken into simpler parts which do give a blue color when boiled with ninhydrin. The question next arose as to whether or not the regular substrates which gave positive results could not have been made negative by further washing and thus freeing the substrate from the materials dealt with above. To demonstrate this point, tests were made on substrates at various stages of washing. The results were as shown in Table 9.

TABLE 9.—Effect of Progressive Washing of Material Used for Substrates

Tissue	No. of Washings	Donor of Serum	Results of Tests		
			Tissue and Serum	Tissue Alone	Serum Alone
Kidney	1	Black	+ + +	+	—
Kidney	1	Terrier	+ + +	—	—
Kidney	1	Collie	+ + +	—	—
Kidney	2	Black	+ +	—	—
Kidney	2	Terrier	+ +	—	—
Kidney	2	Collie	+ +	—	—
Kidney	3	Black	+ +	—	—
Kidney	3	Terrier	+ +	—	—
Kidney	3	Collie	+ ?	—	—
Kidney	5	Black	+	—	—
Kidney	5	Terrier	+ +	—	—
Kidney	5	Collie	+ ?	—	—
Kidney	7	Black	+ +	—	—
Kidney	7	Terrier	+ +	—	—
Kidney	7	Collie	+	—	—
Kidney	12	Sheep-dog	—	—	—
Kidney	12	Black	—	—	—
Kidney	12	Collie	—	—	—
Spleen	5 to 7	Terrier	+ +	—	—
Spleen	5 to 7	Collie	+	—	—
Spleen	5 to 7	Sheep-dog	+	—	—
Spleen	10 to 12	Sheep-dog	—	—	—
Spleen	10 to 12	Collie	—	—	—
Spleen	10 to 12	Black	—	—	—
Muscle	5 to 7	Terrier	+ + +	—	—
Muscle	5 to 7	Collie	+	—	—
Muscle	5 to 7	Sheep-dog	+	—	—
Muscle	10 to 12	Collie	+ +	—	—
Muscle	13 to 15	Collie	+	—	—
Muscle	13 to 15	Black	+	—	—
Muscle	18 to 20	Terrier	—	—	—
Muscle	18 to 20	Sheep-dog	—	—	—
Muscle	18 to 20	Pointer	—	—	—

DISCUSSION AND CONCLUSIONS

It is clear from Tables 1 to 5 that positive Abderhalden tests are frequently obtained with normal serums and substrates prepared from normal blood, brain, kidney, liver, spleen, pancreas, suprarenal, sex gland, thyroid and muscle. From Tables 6 to 9 it is plain that the cause of these positive tests is a material which may be washed out of the substrate, if the washing is continued long enough. Abderhalden's directions for detecting when the substrate is washed enough, however, are not sufficient. The only way to know whether a substrate is fit to use with pathological serums is to have it thoroughly tested with a number of serums from normal persons. If this is not done the results are of small value.

The tests here published show that no definite number of washings can be regarded as sufficient. They must be continued until actual test with normal serums demonstrates the removal of this substance. Abderhalden's directions give no reasons for care in the washing of the substrates, but he does insist[3] on the need for controls of the substrates used for pathological work by testing with normal serums. Unless this be done the results, as Abderhalden states, are entirely worthless. The explanation of the need for this care offered here seems to us to explain many of the discordant results which have been published. We might add that this applies especially to the investigation of reactions with the smaller organs, such as the glands of internal secretion where the amount of material available is small and repeated washing means increased waste.

The published reports of work do not state whether the substrates were always the same in any one series nor how much they were washed, and hence must be studied with caution.

The conclusions may be briefly stated as follows:

1. Using the Abderhalden technic with the Abderhalden reaction, the serums of normal animals frequently show evidence of the presence of ferments capable of digesting various tissues of the body.

2. The material causing the positive Abderhalden reactions between normal serum and normal tissue may be precipitated from the washings by the addition of an equal volume of alcohol.

3. The material causing the positive Abderhalden reactions between normal serum and normal tissue may be completely removed from the substrate if the washing is continued long enough. The number of washings required varies with different individuals and different tissues.

4. The only method which will show whether this material is completely extracted from the substrate is to make a number of Abderhalden tests using a number of normal serums. Until this has been done the substrate cannot be considered fit for use with pathological serums.

THE SPECIFICITY OF CHOLESTERIN WITH SYPHILITIC SERUMS AND OF CHOLESTERIN-REINFORCED HEART ANTIGEN IN THE WASSERMANN REACTION *

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PART I

The intention of the first part of this paper is to present evidence of the safety with non-syphilitic serums and the specificity with syphilitic serums of the cholesterin heart antigen in the Wassermann reaction. The second part of the paper demonstrates that this specificity is due to cholesterin. No attempt will be made to discuss the reasons for this specificity. The work is treated largely from the technical point of view, and thus assumes a general knowledge of the principles and even of the technic of the reaction on the part of the reader.

This paper is based on one thousand Wassermann reactions which were done on the serums of eight hundred different persons, and in addition on eighty-seven Wassermann reactions on the spinal fluid of the same persons. All of these cases have been under observation in this hospital. Since, here, the Wassermann reaction is a routine laboratory test on all medical cases, about three-fifths of the cases were on the medical service under Dr. Christian's observation; likewise on the neurological division of the surgical service the Wassermann reaction is a routine test and, consequently, about one-fifth of the patients were under the observation of Dr. Cushing; the remaining fifth of this number were chiefly from the out-patient department, although a few were from the general division of the surgical service.

I shall not discuss the different antigens which have been utilized in the Wassermann reaction, but will briefly outline the development of the cholesterin antigen. Many independent observations by a great many workers[1] had shown that Wassermann "antigen" was presumably of the nature of a lipoid. Following this assumption several investigators[2] tried different lipoids, and lecithin was used most frequently.

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* From the Medical Clinic of the Peter Bent Brigham Hospital, Boston, Henry A. Christian, Physician-in-Chief.

[1] Lansteiner, Müller and Pötze: Wien. klin. Wchnschr., 1907, xx, 1565. Porges and Meier: Berl. klin. Wchnschr., 1908, xxi, 731. Levaditi and Yamanouchi: Compt. rend. Soc. de biol., 1907, lxiii, 740.

[2] Sachs, H., and Altmann, K.: Berl. klin. Wchnschr., 1908, xxi, 494. Sachs, H. and Rondoni, P.: Ztschr. f. Immunitätsforsh., 1909, i, 132.

Browning, Cruickshank and MacKenzie[3] found that mixtures of lecithin and cholesterin gave a particularly specific reaction with syphilitic serums. The next notable advance in the preparation of antigens was the suggestion of Hans Sachs[4] to combine the alcoholic extracts of animal organs with cholesterin. The addition of cholesterin to extracts of organs was found by Sachs to endow them, however unsatisfactory alone, with properties equal to those possessed by the best syphilitic extracts. McIntosh and Fildes[5] demonstrated that an antigen prepared from human heart and cholesterin showed a notable improvement on the others, in that a much higher percentage of positive results was obtained in undoubted syphilis, while no tendency toward non-specificity was observed.

Recently, Swift and I[6] demonstrated that a 0.4 per cent. cholesterin was a safe and the optimum amount to be used. Also, we concluded that a 0.4 per cent. cholesterinized alcoholic extract of human heart made a constant and a more sensitive antigen than did a fetal syphilitic liver or a similarly made cholesterinized alcoholic extract of the heart or liver of other species, with the exception of the heart of the guinea-pig, which was apparently of the same value. Kolmer, Laubaugh, Casselman and Williams,[7] working independently of us along similar lines and at the same time very shortly afterward published their results confirming our findings.

TECHNIC

On account of the various improvements and modifications which do not enjoy universal recognition, different workers employ different technics, and consequently a certain diversity of result is inevitable. As a result of the variety of technic, the mere statement that a particular Wassermann reaction is negative is insufficient; before the value of such an observation can be appreciated, it is essential to know the methods by which it has been arrived at, and the general results which these methods usually yield. Therefore, I shall first describe my technic rather fully. All quantities of the various reagents are one-half the amounts originally described by Wassermann, Neisser and Bruck.

Complement.—Guinea-pig serum is used to supply the complement. Each day the reactions are performed, two or more guinea-pigs, after being stunned, are bled from the throat into sterile Petri dishes in which the blood clots, and in the interval of one or two hours sufficient serum separates from the clot. A portion of this is made up in a 10 per cent. dilution with saline for titration.

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[3] Browning, C. H., Cruickshank, J., and Mackenzie, I.: Biochem. Ztschr., 1910, xxv, 85; Jour. Path. and Bacteriol., 1910, xiv, 484.

[4] Sachs, H.: Berl. klin. Wchnschr., 1911, iii, 2066.

[5] Fildes, P., and McIntosh, J.: Brain, 1913, xxxvi, part 11.

[6] Walker, I. C., and Swift, H. F.: Jour. Exper. Med., 1913, xviii, 75.

[7] Kolmer, F. A., Laubaugh, E. E., Casselman, A. J., and Williams, W. W.: Practical Studies on the So-Called Syphilis "Antigens," with Special Reference to Cholesterinized Extracts, The Archives Int. Med., 1913, xii, 660.

Blood-Corpuscles. — These are obtained from the sheep at a slaughter-house, the blood being caught in a sterile bottle containing glass beads, and this bottle is shaken from three to five minutes to defibrinate the blood. This sheep blood is obtained fresh each day of the reactions, is washed four or five times with more than an equal volume of saline, the time that the centrifuge is running for the last washing is always accurately noted, and the centrifuge is always running at the same speed for this last washing each day, so that each day the amount of corpuscles is approximately the same. For the reactions 0.5 c.c. of a 5 per cent. suspension in saline is used.

Saline Solution.—Pure crystallized sodium chlorid and distilled water in the strength of 0.85 per cent. is used.

Amboceptor.—The sheep-rabbit amboceptor was used. By using diminishing quantities of the amboceptor with one unit (0.25 c.c. of a 10 per cent. dilution) of complement, 0.5 c.c. of a 5 per cent. sheep cell-suspension and saline up to the standard volume (2.5 c.c.), the minimum hemolytic dose (M. H. D.) of the amboceptor was determined. Having found that 0.0008 c.c. of this amboceptor was the M. H. D. with one unit of complement, by working with dilutions so arranged that 0.25 c.c. of the dilution contained 0.0008 c.c. of amboceptor, another dilution slightly more, and a third slightly less than this amount, it was found that 1 to 350 was the proper dilution of amboceptor (see Protocol 1). This titration was repeated with several specimens of pooled complement until a constant optimum was determined, and this optimum dilution is considered the standard. Two units of amboceptor diluted to 0.5 c.c. with saline were used to sensitize each 0.5 c.c. of the 5 per cent. sheep cell suspension for the reactions.

Standardization of the Hemolytic System.—A most important step in the Wassermann reaction is the accurate titration of the hemolytic system, and this should be done each time before performing the reactions. Occasionally sheep corpuscles are resistant to hemolysis; when such is the case I discard them and use the previous day's cells. Occasionally the complement is weaker than 10 per cent.; if so I discard it, but if it is stronger than 10 per cent., I use it in the proper strength as determined by titration. Therefore, to recapitulate, the constituents of the hemolytic system are: amboceptor made up with saline in the dilution as standardized so that 0.25 c.c. of dilution represents one unit; a sample portion of the complement made up with saline in a 10 per cent. dilution, 0.25 c.c. representing one unit; a 5 per cent. suspension of sheep corpuscles, 0.5 c.c. being the standard amount, and sufficient saline to each tube to make the total volume the same, which should be 2.5 c.c., the volume in the Wassermann test. Both amboceptor and complement are titrated in varying amounts, and the cells, the complement and cells, and the amboceptor and cells are also controlled (see Protocol 1).

Protocol 1 shows the method of standardization of the amboceptor after the M. H. D. has been determined and diluted so that 0.25 c.c. contains one unit of amboceptor, and the protocol also represents the daily titration of the hemolytic system except that only one dilution of the amboceptor (the standardized dilution) is used. Having determined the M. H. D. of complement, the total guinea-pig serum is then diluted so that 0.5 c.c. of the dilution represents two units of complement, which amount is used in the reactions.

PROTOCOL 1.—Method of Standardization of the Amboceptor

Tube No.	Amount of Complement			All made up to 2.5 c.c. Incubation one hour at 37 C.	Dilution of Amboceptor *			
	Amboceptor Dilution c.c.	Guinea-Pig Serum 10 Pct., c.c.	Sheep Cells 5 Pct., c.c.		1:250	1:300	1:350	1:400
1	0.5	0.5	0.5		C. H.	C. H.	C. H.	C. H.
2	0.4	0.5	0.5		C. H.	C. H.	C. H.	C. H.
3	0.3	0.5	0.5		C. H.	C. H.	C. H.	C. H.
4	0.25	0.5	0.5		C. H.	C. H.	C. H.	C. H.
5	0.2	0.5	0.5		C. H.	C. H.	C. H.	C. H.
6	0.1	0.5	0.5		C. H.	N. C. H.	N. C. H.	Mod. H.
7	0.25	0.4	0.5		C. H.	C. H.	C. H.	C. H.
8	0.25	0.3	0.5		N. C. H.	C. H.	C. H.	C. H.
9	0.25	0.25	0.5		Mod. H.	N. C. H.	C. H.	N. C. H.
10	0.25	0.2	0.5		Sl. H.	N. C. H.	N. C. H.	Sl. H.
11	0.25	0.1	0.5		Sl. H.	Mod. H.	Mod. H.	Sl. H.
12	0.5	....	...		0			
13	....	0.5	...		0			
14	....	....	0.5		0			

* C. H. = complete hemolysis; N. C. H. = nearly complete hemolysis; Mod. H. = moderate hemolysis; Sl. H. = slight hemolysis; 0 = no hemolysis.

Human Serum.—This is inactivated at 56 C. for one-half hour and 0.1 c.c. is used in each test-tube, and 0.05 c.c. in a control-tube for natural sheep amboceptor, and 0.2 c.c. in the anticomplementary control-tube.

Preparation of Antigen.—Human heart, free from gross fat, was weighed, finely minced and placed in absolute alcohol in the proportion of 1 gm. of tissue to 10 c.c. of absolute alcohol. Extraction was carried on in the incubator at 37.5 C. for from seven to ten days, the bottles being shaken several times daily. The bottles were allowed to stand at room temperature for a day or two, and then filtered. Usually after two or three days it was necessary to filter again, and the extract was now kept at room temperature as a stock solution. As often as necessary, 25 c.c. were taken from this stock solution and to it 0.1 gm. of cholesterin was added (a 0.4 per cent. cholesterin solution) and placed in the incubator over night, during which time the cholesterin would go into solution, but if it did not dissolve, a little shaking and a few more hours of incubation would be sufficient to dissolve it. This cholesterinized extract may be kept with safety for a month, after which time it is liable to become slightly anticomplementary, and a new portion of the stock solution should be cholesterinized.

Each day the reactions are done the antigen for the test-tubes is made as follows: to 9 c.c. of 0.85 per cent. saline is added, drop by drop with constant shaking, 1 c.c. of the 0.4 per cent. cholesterin alcoholic extract, thus making a 1 : 10 emulsion and 0.5 c.c. of this 1 : 10 emulsion of antigen is used in each test-tube.

PROTOCOL 2.—Wassermann Reaction

FRONT TUBES

0.1 c.c. patient's serum.	0.1 c.c. patient's serum.
0.5 c.c. complement (2 units).	0.5 c.c. complement (2 units).
0.5 c.c. antigen No. 1 (1:10 emulsion).	0.5 c.c. antigen No. 2 (1:10 emulsion).
0.4 c.c. saline.	0.4 c.c. saline.

BACK TUBES

0.2 c.c. patient's serum.	0.05 c.c. patient's serum.
0.5 c.c. complement.	0.25 c.c. complement (1 unit).
0.8 c.c. saline.	0.5 c.c. cells.
	1.7 c.c. saline.

Incubation one hour at 37.5 C. in water bath. Then 1 c.c. of sensitized cells is added to each tube except that one already containing cells. Incubation is again carried on for another hour in water bath at 37 C., after which time the results are noted.

In Protocol 2 the front tubes are the test-tubes and are in duplicate with the exception of different antigens; that is, the same dilution of antigen is used, but made from two different hearts and the cholesterin has been added to each heart extract at different dates. The back tubes serve as controls, the one containing 0.2 c.c. of patient's serum is the anticomplementary control. The other tube contains one-half the amount of serum and complement, no antigen and no amboceptor and the standard amount of cells; this serves as the control for natural sheep amboceptor which may be present in the serum. The reason for using two antigens and the natural sheep amboceptor control will be mentioned later. A known positive and a known negative serum are always used as controls and each antigen is controlled. Complete inhibition of hemolysis is represented by four pluses (+ + + +), 75 per cent. inhibition by + + +, one-half inhibition by + + and less than this by a single +. These varying amounts of inhibition are determined by centrifuging, but even then there is considerable variation.

ADVANTAGES OF THIS ANTIGEN

There are three chief advantages in such an antigen; one, the ease with which it is obtained and made; another, its constant properties when made from different hearts; the third, its superior antigenic properties with syphilitic serums with no tendency toward non-specificity.

The ease of obtaining and making this antigen has been mentioned in a preceding paragraph. The alcoholic extract may be kept as a stock solution for at least six months, and to portions of this stock solution cholesterin may be added in 0.4 per cent. strength at intervals of four or five weeks, or oftener if necessary.

PROTOCOL 3.—Difference in Antigenic—

Antigen Used	Serum of Miss W.		Serum of Mr. S.		Serum of Mr. D.	
	0.1 cc.	0.05 c.c.	0.1 cc.	0.05 c.c.	0.1 cc.	0.05 c.c.
Heart cholesterin 1:6 emulsion	++++	++++	++++	+	++++	++++
Heart cholesterin 1:12 emulsion ...	++++	+++	+	—	++++	++++
Liver cholesterin 1:6 emulsion	++++	++++	+++	—	++++	++++
Liver cholesterin 1:12 emulsion ...	+++	+	—	—	++++	++++
Liver alone 1:6 emulsion	—	—	—	—	++++	+
Liver alone 1:12 emulsion	—	—	—	—	—	—

That different hearts yield antigens of the same antigenic and anticomplementary properties has been demonstrated by McIntosh and Fildes. These workers used twenty-four different human hearts, and in addition to the above found that the addition of cholesterin rendered them invariable, so that a quantitative estimation may be carried out with different reagents and different antigens at different times with a very small margin of error. In this present work I have used ten different human hearts from patients who had died from the following causes; pneumonia, diabetic coma, uremia, general carcinomatosis, sarcoma of skull, gumma of brain and liver, carcinoma of head of the pancreas with cholemia, myxedema with chronic myocarditis, and two from syphilitic aortitis. All of these hearts had practically the same antigenic and anticomplementary properties. Furthermore, quantitative estimations of their antigenic properties with different syphilitic serums and different reagents were practically the same and were always constant for the same antigen from day to day.

McIntosh and Fildes demonstrated the superior antigenic properties of the cholesterin heart antigen over other antigens with manifest syphilitic serums from primary, secondary and tertiary cases. This advantage was most apparent in syphilis of the central nervous system, including parasyphilis in which group of cases the syphilitic liver antigen has inferior antigenic properties.

Swift and I found the same advantages were apparent when we compared this antigen with those considered to be the best in use in Germany. For this work I was unable to obtain a congenital syphilitic liver, but I did make an antigen from several gummata of a liver in which the spirochetes were demonstrated, using the alcoholic extract made in the same method as the heart extract. A series of Wassermanns using graded amounts of serums from known syphilitic patients was performed with the following antigens: a 0.4 per cent. cholesterinized alcoholic human heart extract, an alcoholic extract of the liver gumma and a 0.4 per cent. cholesterinized alcoholic extract of the liver gumma. Protocol 3 demonstrates the difference in antigenic properties of the three different antigens.

—Properties of Three Different Antigens

Serum of Mrs. K.		Serum of Mrs. A.		Serum of Mr. W.		Serum of Mrs. J.	
0.1 cc.	0.05 c.c.	0.1 cc.	0.05 c.c.	0.1 cc.	0.05 c.c.	0.1 cc.	0.05 c.c.
++++	++++	++++	++	++++	+++	++++	++++
++++	++++	++++	+	+++	+	++++	+
++++	+	++++	+	+++	+	++++	+
++	—	+	—	+	—	+	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—

From the Protocol 3 it is evident that the cholesterin heart antigen is the most sensitive, the gummatous liver antigen the least sensitive — in fact, it gave no fixation with some clinically syphilitic serums — and the cholesterinized gummatous liver, although made much more sensitive by the addition of the cholesterin, was still less sensitive than the heart cholesterin antigen.

DISADVANTAGES OF THIS ANTIGEN

Careless workers and those who are untrained in and unfamiliar with the complexity and the technic of the Wassermann reaction may obtain false positive reactions in non-syphilitic cases. This can hardly be considered a disadvantage, since only trained laboratory men should do this test, and if those laboratories which do employ untrained persons — and we know such is the lamentable fact in this country and in Germany — can be stimulated to employ trained persons a noteworthy result will be accomplished, since the Wassermann reaction is one of the most, if not the most, specific laboratory test that is known at the present time.

The hemolytic system should be accurately and carefully titrated each time the reactions are done, since variations in the amount of amboceptor used cause variations in the amount of complement necessary and cholesterin binds complement, consequently too small an amount of complement may be bound partially by the cholesterin and an insufficient amount may be free to complete the hemolytic system in certain negative serums.

The cholesterinized alcoholic heart extract may become slightly anticomplementary after four or five weeks. This fact is quickly recognized by controlling the antigens without serums, and with known negative serums each time the reactions are done and by using extracts to which cholesterin has been added at different intervals.

Occasionally a certain specimen of cholesterin will be anticomplementary. Out of seven different lots of supposedly C. P. cholesterin only one lot has been anticomplementary, and this was definitely proved to be unsatisfactory. The cholesterin used in this work was purchased from Eimer and Amend and from Merck and Company, and always in small amounts in order to obtain several different samples.

NO NON-SPECIFIC FIXATION WITH NON-SYPHILITIC SERUMS

In this series of 800 different persons there were 667 who gave a negative Wassermann reaction with the 1 : 10 emulsion. In none of these cases was there a question of syphilis, and since, as previously stated, all of the cases were in patients at this hospital, they consequently were carefully observed. The diagnosis of the prominent condition in the majority of these patients is represented in the table following; those diagnoses which apply to only a very limited number of cases are grouped under miscellaneous:

Chronic cardiac disease	55	Ulcer of stomach or duodenum	8
Chronic nephritis	40	Migraine	12
Tuberculosis	30	Varicose veins	6
Cirrhosis of liver	9	Rectal abscess	5
Typhoid fever	12	Pleurisy	13
Pneumonia	25	Goiter	7
Epilepsy	18	Tonsillitis	13
Brain tumor	37	Diabetes mellitus	17
Trigeminal neuralgia	15	Tabes	4
Acromegaly	10	Neurasthenia	6
Chronic salpingitis	11	Observation	8
Malignant disease	16	Hysteria	6
Spinal cord sclerosis	8	Hernia	8
Chronic arthritis	14	Disease of eye	6
Syphilis cured	14	Cystitis and pyelitis	9
Anemia	8	Cholelithiasis	6
Skin disease	20	Normal persons	50
Metrorrhagia	6	Miscellaneous	127
		Total	667

It might be well to call attention to the few following cases in the foregoing table. The fourteen patients who gave a history of syphilis had had their chancre years ago, and following this they had very intensive treatment. In these cases the Wassermann reaction was repeatedly negative and two of the cases came to necropsy and no evidence of syphilis was made out. All of the diabetic patients had acetone and diacetic acid and large amounts of sugar in the urine when the blood was taken for the Wassermann reaction, and on several of these the reaction was repeated when they became sugar free. Three of the cases were in diabetic coma, four were in uremia and several others were *in extremis* from other causes.

In using 0.5 c.c. of the 1 : 10 emulsion of this antigen, we are taking only one-fourth of the anticomplementary dose, which is well within the limit of safety, since most workers state that one-third of the anticomplementary dose is safe. Now, this same antigen made up in a 1 : 6 emulsion and using 0.5 c.c. for each test is nearly twice as strong as the 1 : 10 emulsion, and is too near for safety to the anticomplementary dose. Nevertheless, in order to determine how near this was to the non-specific fixation limit, in 400 different serums, 0.5 c.c. of 1 : 6 emulsion was employed in an extra test-tube. These 400 cases were in two series, one of 186 consecutive serums, and the other of 214 consecutive serums. Of these 400 cases, all of which gave a negative reaction with the 1 : 10 emulsion, 380 also gave a negative reaction with the 1 : 6 emulsion. Of the 20 cases which were not entirely negative, 12 gave a questionable reaction, that is, very slight inhibition of hemolysis; on centrifuging the tubes a very few cells were evident; 8 serums gave a + + reaction, that is, 50 per cent. or less inhibition of hemolysis. The 12 cases giving such a very slight degree of inhibition may be safely set aside, since no consequence would be attached to such a slight degree of inhibition, and mechanical errors could easily account for such. Therefore in only 8 cases, or 2 per cent., which gave a + + reaction could any one be suspicious of non-specific fixation, and even then such reactions are always considered as negative unless the particular case is understood.

Another test-tube containing 0.2 c.c. of patients serum was used with the 1:10 emulsion in 400 consecutive cases. In none of these cases was there the slightest inhibition of hemolysis with the 0.2 c.c. of serums when there was none with the 0.1 c.c. of serums.

Therefore, in such a large series of cases, since the 1 : 10 emulsion gave no non-specific fixation with either 0.1 c.c. or 0.2 c.c. of serum, and since the 1 : 6 emulsion, which is theoretically too near the anticomplementary dose, gave only 2 per cent. non-specific fixation (and this indeed was only slight in amount) it would seem that the 1 : 10 emulsion of this antigen with 0.1 c.c. of serum was certainly safe and sane.

Among the 87 different spinal fluids tested, 74 were negative, and in these the cell-count did not exceed 5 per cubic millimeter, and the globulin reaction was negative with the exception of four cases of tuberculous meningitis, the diagnosis of which was verified at necropsy. The majority of the patients in these negative cases were operated on by Dr. Cushing and no syphilitic process was evident; many of the others were from cases of coma, uremia, etc. In addition to those above mentioned, there were several ventricular fluids and brain-cyst fluids obtained from operations by Dr. Cushing, all of which were negative. With many of these the 1 : 6 emulsion of the antigen was also used with the same negative results as with the standard 1 : 10 emulsion. The technic of the spinal fluid Wassermann differed from that of the serum Wassermann in that larger quantities of spinal fluid were used and the amounts were graded. Seven tubes were used for each spinal fluid; each of six tubes containing, respectively, 0.5 c.c., 0.4 c.c., 0.3 c.c., 0.2 c.c., 0.1 c.c., 0.05 c.c. and a control-tube containing 1.0 c.c. for anticomplementary control; to the six tubes with diminishing amounts of fluid were added 0.5 c.c. of the 1 : 10 emulsion of the antigen, 0.5 c.c. complement, and saline sufficient to make all tube contents equal, and after one hour incubation, 1 c.c. of sensitized cells was added and incubation was carried on for another hour. By using this graded system considerable information was derived concerning prognosis, diagnosis and result of treatment.

FIXATION WITH SYPHILITIC SERUMS

I am aware of the fact that the diagnosis of syphilis on the strength of a positive Wassermann reaction without any history of infection is open to much criticism. Although I believe that a positive Wassermann reaction, properly done, with or without a history of infection, does mean syphilis (barring certain conditions, as florid scarlatina and leprosy, none of which should be clinically confused with syphilis), nevertheless, for the benefit of a doubt in the mind of the reader, the facts in support of the diagnosis of syphilis apart from a positive test will be given in some detail. Improvement clinically and symptomatically and a decrease in the intensity of the reaction under intravenous salvarsan and intramuscular mercury treatment will be considered important evidence in favor of a syphilitic process. Realizing that the burden of the proof "that this cholesterin heart antigen gives no non-specific fixation" lies in the proof that all the positive reactions were of a syphilitic nature compels me to go into considerable detail in some cases, and I trust that the reader who may not be skeptical will be patient.

Those serums which gave a positive Wassermann with the 1 : 10 emulsion of the antigen were subjected to a titration for their "fixing unit." By "fixing unit" is meant the smallest amount of serum which would give complete inhibition of hemolysis in the presence of the standard amount of complement, antigen and required saline. This quantitative test was performed as follows: The maximum quantity of serum used was 0.1 c.c., and this quantity was halved five times, so that in a series of six tubes there was respectively 0.1 c.c., 0.05 c.c., 0.025 c.c., 0.012 c.c., 0.006 c.c. and 0.003 c.c. in the various tubes; therefore, the amount of serum was varied, but the amount of complement and antigen was kept constant. If it was found that 0.012 c.c. gave complete inhibition, but 0.006 c.c. did not, then 0.012 c.c. was the "fixing unit," and from now on the capitals F. U. will represent "fixing unit."

In this series of 800 cases, there were 110 persons who gave complete inhibition of hemolysis with the 1 : 10 emulsion of the antigen. Of this number, 39 gave a definite history of a chancre which was followed by medication. These 39 cases will be dismissed with no further comment, since the chancre suffices to explain the positive Wassermann. The remaining 71 cases are tabulated below, with the diagnosis of the existing condition, and confirmatory evidence if present will be given in each case; these gave no history of chancre.

It would seem that the above-mentioned cases showed sufficient evidence to substantiate the probability of a syphilitic infection, and that the small amount of serum (F. U.) required to give complete inhibition of hemolysis was, in doubtful cases, too small to think of non-specific fixation; furthermore, the symptoms in so many of the cases which might possibly be questioned were relieved by salvarsan injections. Although the fact is not mentioned above, the "fixing unit"

No. of Cases	Diagnosis	
6	Rash	All relieved by salvarsan intravenously.
3	Tabes dorsalis	All had positive Wassermann, positive globulin, increased cell-count in spinal fluid.
2	General paresis	Same as for tabes.
7	Aneurysm	Substantiated by Roentgen-ray.
4	Dilated aorta	One patient had had six miscarriages; one had one miscarriage and two monoplegias; one had positive Wassermann elsewhere; one showed symptomatic improvement with potassium iodid medication. All were substantiated by Roentgen-ray.
5	Periostitis	Symptoms and signs disappeared after many intramuscular injections of salicylate of mercury. All verified by Roentgen-ray.
4	Gummata	Two verified by operation and all disappeared under treatment.
1	Cerebrospinal Syphilis	Wassermann and globulin positive, cells increased in spinal fluid.
5	Congenital syphilis	Definite stigmata.
4	Ulcers	All were healed by intramuscular mercury and by salvarsan.
3	Aortitis	Physical signs of; the wife of one man had two still-born and no living children.
8	Uterine *	One with two miscarriages and no children; one with three still borns and no children; one with three miscarriages and no children; one with five miscarriages and no children; one with three miscarriages and husband had positive Wassermann; one with two still borns at seventh month and no children; two with one miscarriage, several D. and C. operations and no children.
2	Dementia	Positive Wassermanns elsewhere, and repeatedly positive here.
4	Vomiting	All four had attacks of recurrent vomiting, and dizziness and all were relieved immediately by intravenous salvarsan.
2	Vertigo	Vertigo and headaches for year or more, relieved by several salvarsan injections.
1	Paroxysmal hemoglobinuria	Relieved by salvarsan. Ninety per cent. of these cases are syphilitic.
1	Laryngitis	Cured by treatment with mercury.
1	Epilepsy	Attacks stopped after salvarsan.
1	Neuralgia	Relieved by salvarsan; patient gave indefinite history of infection.
1	Pellagra	Relieved by salvarsan and Wassermann became negative after ten intravenous treatments.
1	Diabetes insipidus	
1	Pelvic disease	Admitted exposure for three years, salvarsan relieved symptoms and Wassermann became negative after several salvarsans.
2	Pneumonia	One a woman who never had borne children and the Wassermann was repeatedly positive. F. U., 0.003 c.c. One, a man, whose wife never had borne children, Wassermann repeatedly positive. F. U., 0.025 c.c.
1	Alcoholic	Wassermann positive with syphilitic liver antigen, repeatedly positive here. F. U., 0.05 c.c.
1	Diabetes mellitus	One miscarriage followed by no healthy children. F. U. 0.003++. Wassermann also positive with syphilitic liver antigen.

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* Whitehouse[8] recognizes a syphilitic fibrosis of the uterus, a chronic metritis and fibrosis, with a positive Wassermann in the serum.

8. Whitehouse, B.: Jour. Obset. and Gyn. Brit. Emp., 1914, xxv, 1.

in all of the questionable cases was greatly increased by treatment, so that every two weeks, when the titration test was done, the amount of serum necessary to give complete inhibition increased.

There were twenty-four persons whose serums gave only from 50 per cent. to 75 per cent. inhibition of hemolysis with the 1 : 10 emulsion of the antigens. It is in these cases with a more or less doubtful reaction that the non-specific fixation must be ruled out.

No. of Cases	Diagnosis	
3	Tabes dorsalis	The spinal fluids of all gave a positive Wassermann and globulin and an increase in the cells. Also classical signs.
1	Congenital syphilis	Had had mercury and KI constantly for several years.
6	Tertiary	All had definite chancre followed by intensive treatment.
1	Cerebral	Spinal fluid Wassermann and globulin positive, increase in cells.
1	Secondary syphilis	Had had intensive treatment, came to hospital for salvarsan.
1	Epilepsy	Attacks relieved by potassium iodid.
1	Migraine	Relieved by intramuscular mercury and potassium iodid.
6	Miscarriage	One a woman who had two miscarriages and two still borns; one a woman who had two miscarriages and a congenital child; one a woman who had three miscarriages since a healthy child; one woman who had one miscarriage; one a woman who had seven miscarriages; one a woman who had four miscarriages and two stillborns. All the above cases became negative after intramuscular mercury injections.
1	Cerebral	Had two attacks of monoplegia with aphasia.
1	Salpingitis	Became negative after many intramuscular mercury injections.
1	?	Was diagnosed syphilitic at another hospital.
1	Catarrhal jaundice	No evidence of syphilis and became negative after jaundice cleared.

Although it is a rule that partial inhibition like that in the cases mentioned above should be considered negative or, at the most, only suspicious unless the previous condition of the patient is sufficiently well understood, it would seem that with this antigen a 50 per cent. or 75 per cent. inhibition of hemolysis really meant a pretty definite syphilitic trace. All the above-mentioned cases were repeated at least once and the degree of inhibition varied only slightly, with the exception of the catarrhal jaundice case.

There were thirteen cases with a positive Wassermann in the spinal fluid, and in all the globulin was positive and the cells increased above ten. The diagnosis in these cases was clinically certain.

THE USE OF THE 1:6 EMULSION IN CERTAIN CASES

As previously stated, the 1 : 10 emulsion is used for diagnosis and the 1 : 6 emulsion was used merely to determine in how many cases it might give non-specific fixations. The use of this 1 : 6 emulsion brought out several interesting facts. One fact noted was that several secondary syphilitic cases, when treated until their serums gave a negative reaction with the 1 : 10 emulsion, but still showing from 50 per cent. to 75 per cent. of inhibition of hemolysis with the 1 : 6 emulsion, all became positive again with the 1 : 10 emulsion after an interval of a few weeks without treatment. On the other hand, several secondary syphilitic cases which were treated until they were negative with the 1 : 6 emulsion as well as with the 1 : 10 emulsion have not become positive again at all with either emulsion after several months without treatment. Therefore, we now do not stop treatment in early cases until the serum is negative with the 1 : 6 emulsion.

The same was true in two late stages of syphilis involving the central nervous system, in both of which the serums were negative after intensive treatment, but the spinal fluid was positive with the 1 : 10 emulsion, 0.5 + + + + and 0.4 + + + +. In both these cases, one of early tabes, the other of cerebral syphilis, intraspinal salvarsanized serum was given without other medication. These two patients gradually improved, and after many treatments their spinal fluids became negative with the 1 : 10 emulsion, but they both still had symptoms, although much improved. Treatment was still continued, and when one patient was lost track of the spinal fluid was slightly positive with the 1 : 6 emulsion (0.5 + +), and his symptoms were slight. The tabetic patient is still being treated; his spinal fluid Wassermann is 0.5 + + + and his symptoms are very slight. Since these two cases with a negative spinal fluid Wassermann with the 1 : 10 emulsion still had symptoms and a slightly positive reaction with the 1 : 6 emulsion, it would seem that in central nervous system involvement there was indication for continuance of treatment until the spinal fluid Wassermann became negative to the 1 : 6 emulsion.

In two cases of tabes, one with a Charcot knee, the other with gastric crises, the blood Wassermann was negative with the 1 : 10 emulsion, but with the 1 : 6 emulsion one serum gave a + + reaction, the other a + + + + reaction. From these two cases one would infer that fixation with the 1 : 6 emulsion should invite careful consideration of a syphilitic process.

In five persons each of whom had a chancre of from one to three weeks' duration, the serums of all were negative with the 1 : 10 emulsion, but with the 1 : 6 emulsion four gave complete inhibition, and one gave 50 per cent. inhibition of hemolysis. Here again inhibition of hemolysis with the 1 : 6 emulsion would indicate a syphilitic process.

Furthermore, it was noted that when there was present in the serum much natural sheep amboceptor, the tube with 0.2 c.c. of serum and the 1 : 10 emulsion showed complete hemolysis, and the tubes containing 0.1 c.c. of serum and the 1 : 10 emulsion showed partial hemolysis, so that a questionable reaction resulted, the 1 : 6 emulsion with 0.1 c.c. of serum showed no hemolysis. In such a case the 1 : 6 emulsion threw the evidence of the questionable reaction with the 1 : 10 emulsion toward the side of a positive reaction.

With the twenty-four serums which gave partial inhibition, as reported above, with the 1 : 10 emulsion, there was from 75 per cent. to complete inhibition with the 1 : 6 emulsion, and all except one case were pretty surely syphilitic.

Therefore, from the preceding statements, it would seem that the 1 : 6 emulsion might well be used as a sort of control over the 1 : 10 emulsion, but that its value in diagnosis should be carefully considered; its value as an index to further treatment is great.

PART II

TITRATION METHODS SHOWING THE SPECIFICITY OF CHOLESTERIN WHEN USED TO REINFORCE AN ANTIGEN IN THE WASSERMANN REACTION

As previously described, all serums with a positive Wassermann were titrated in diminishing quantities with the constant normal amount of complement and antigen and sufficient saline to equalize the volume in all tubes. This was done frequently to note the improvement by treatment. The 1 : 6 emulsion of the antigen was the strength used for this titration, since it was not affected by the presence of natural sheep amboceptor, and because it was anticipated that in very early cases the serums would give too weak a reaction with the 1 : 10 emulsion to be of any value, and especially because all cases were treated until their serums became negative to the 1 : 6 emulsion. The smallest amount of serum which gave a complete inhibition of hemolysis was called the "fixing unit" or F. U. It was noted that the serums of different untreated persons had a different F. U., and this did not seem to depend on how long the person had had his syphilis. In other words, the amount of serum necessary to give a complete inhibition of hemolysis bore no relation to the stage of the disease, and the serums of different untreated persons in the same stage of the disease showed wide variation in their F. U. These facts are demonstrated in Protocol 4.

In testing the anticomplementary and antigenic properties of these various antigens with known negative and known positive serums, it was noted that although the F. U. of the serums of different persons in the same stage of syphilis varied, the amount of antigen necessary to fix the serums of patients in the same stage remained practically constant. This fact led to further investigation along this line with the serums of persons who could be grouped clinically and by definite histories into primary, secondary and tertiary syphilis. The basis for this classification is as follows: The primary cases were those with a definite hard chancre of from one to three weeks' duration. The secondary cases were those with a rash which followed a hard chancre four to eight weeks later, and those with mucous patches in the mouth following a primary lesion; this group of cases was substantiated by the rapidity of disappearance of signs and symptoms following one or two injections of salvarsan. The tertiary cases were those with a clinical tertiary rash which appeared many months or a few years after the primary or secondary manifestations; these cases required from six to eight injections of salvarsan before the rash entirely faded; also in this group were placed gummatous lesions, and large broken-down ulcers which followed, by a period of years, a definite hard chancre, or unquestionable secondary manifestations. In general, syphilis would more correctly be referred to as early and late manifestations, but the preceding classification more nearly assumes the purpose of this paper.

PROTOCOL 4.—F. U. in Relation to Stage of Disease

Name	Stage of Disease	F. U. in c.c.
Mr. A.	Tertiary rash	0.012
Mrs. A.	Tertiary rash	0.025
Mr. H.	Tertiary rash	0.05
Mr. K.	Tertiary rash	0.025
Mrs. O.	Tertiary rash	0.012
Mrs. K.	Secondary rash	0.05
Mr. P.	Secondary rash	0.1
Miss McC.	Secondary rash and mucous patches	0.006
Mr. McG.	Secondary rash	0.003
Mr. I.	Congenital	0.012
Mr. F.	Congenital	0.025

The method used in this work is as follows: After the F. U. of the serum was determined, this was kept constant together with the complement (2 units) and the antigen was varied; diminishing amounts of antigen were used in successive tubes or, in other words, the antigen was titrated in diminishing amount with a constant amount of serum and complement. A series of eight tubes was used and to each tube containing the F. U. of serum and 2 units of complement was added respectively 0.5 c.c., 0.4 c.c., 0.3 c.c., 0.2 c.c., 0.1 c.c.. 0.05 c.c., 0.025 c.c.

of antigen, and sufficient saline to make the volume of all tubes equal. In the last three tubes the quantity of antigen, which was too small to be accurately measured, was obtained by diluting 0.4 c.c. of the 1:6 emulsion with 2 c.c. of saline, and using 0.3 c.c., 0.15 c.c. and 0.07 c.c., respectively. After an hour incubation in the water-bath at 37.5 C., 1 c.c. of sensitized cells was added to each tube and incubation was carried on another hour, at the end of which time the results were noted. The tube containing the least amount of antigen which showed complete inhibition of hemolysis was considered the result or the F. U. of antigen necessary to bind the F. U. of serum. This end-result was read after centrifuging the tubes or after allowing them to stand over night in the ice-box. Each serum always had an anticomplementary control-tube. The results obtained with several serums from typical cases is shown in Protocol 5

From Protocol 5 it is evident, as in the preceding protocol, that the amount of serum necessary to give complete fixation has no bearing on the stage of the disease; on the other hand, there is, however, a definite relation between the amount of antigen necessary to give fixation and the stage of the disease. Those serums from patients in a certain stage of the disease required a similar definite amount of antigen which showed no variation for that particular stage, although the amount of serum varied widely. It would seem that by this method one could determine whether a syphilitic rash was an early or a late manifestation, provided the patient had had little or no treatment. Several other cases with rashes, but without a definite history of infection, were by this method grouped either as early or late manifestations and the proper grouping was confirmed later by treatment; that is, if the rash was resistant to treatment it was considered a tertiary or late rash, if it yielded readily to one or two treatments it was considered a secondary or early rash. Furthermore, one is able by this method in mildly treated or non-treated cases to get a fair idea as to whether the patient has had syphilis a few weeks, a few months or a period of years. If this should hold true in a large series of cases, it would be of more than passing interest, especially in medicolegal and insurance cases.

Another interesting point is that cases with a syphilitic dilatation of the aorta, commonly called a dilated arch, as evidenced by physical signs and by Roentgen-ray examination, required a considerable larger amount of this antigen to give complete fixation than did cases with a pronounced aneurysm of the aorta. This is in keeping with the pathology of such lesions, since dilatation of a moderate degree must precede aneurysm and the progress of the lesion from one of slight dilatation to a definite aneurysm probably requires a fairly long period of

PROTOCOL 5.—F. U. IN SEVERAL TYPICAL CASES

Name	Stage of Disease	F. U. in c.c. (++++)	
		Serum	Antigen
PRIMARY			
Mr. R.	Chancre three weeks' duration	0.1	0.5
Miss R.	Chancre one week's duration	0.1	0.5
Mr. M.	Chancre three weeks' duration	0.1	0.5
Mr. S.	Chancre three weeks' duration	0.1	0.5
SECONDARY			
Mr. McG.	Chancre two months ago, sore throat, falling out of hair, rash at present	0.003	0.3
Miss McC.	Chancre, mucous patches in mouth, rash at present	0.006	0.3
Mrs. B.	Chancre six months ago, no secondaries	0.1	0.3
Mr. Z.	Chancre, three months ago, rash one month ago	0.05	0.3
Mr. P.	Chancre, uveitis	0.1	0.3
Mrs. K.	Chancre, secondary rash	0.05	0.3
B. C. H.	Chancre, eight weeks' duration	0.1	0.3
Mr. J.	Chancre, six weeks' duration, beginning rash	0.003	0.4
LATE			
Mr. H.	Chancre four years ago, tertiary rash at present	0.05	0.05
Mr. K.	Chancre eight years ago, tertiary rash at present	0.025	0.05
Mr. P.	Chancre five years ago, tertiary rash at present and ulcer	0.025	0.025
Mr. L.	Chancre nine years ago, general glandular enlargement	0.006	0.025
Mr. G.	Chancre (?) years ago.	0.025	0.025
Mrs. S.	Chancre ten years ago, ulcers in throat at present	0.012	0.025
Miss B.	Chancre five years ago, ulcer in throat now	0.025	0.05
Mr. M.	Chancre three years ago, rash now	0.1	0.05

time; in other words, it is a slow, gradual process. Unfortunately, in none of my cases could I obtain a history of chancre, and consequently I was unable to determine how long the patient had syphilis; therefore, possibly, this would be an exception to the preceding statement that, in general, one could determine by this method whether a patient had had syphilis weeks, months or years. I feel certain, though, that it does apply to syphilic rashes. Protocol 6 represents the fixation in cases with a dilated arch and those with an aneurysm.

PROTOCOL 6.—Fixation in Cases with Dilated Arches and Aneurysms

Name	Stage of Disease	F. U. in c.c. (++++)	
		Serum	Antigen
Miss W.	Dilated arch (Roentgen-ray)	0.006	0.4
Mr. S.	Dilated arch (Roentgen-ray)	0.1	0.4
Mr. J.	Diffuse dilatation of aorta (necropsy)	0.05	0.3
Mr. F.	Aneurysm (Roentgen-ray)	0.025	0.2
Miss G.	Aneurysm (Roentgen-ray)	0.025	0.1
Mr. S.	Aneurysm (Roentgen-ray)	0.025	0.2
Mr. M.	Aneurysm, ruptured (necropsy)	0.003	0.1
Mr. N.	Aneurysm (Roentgen-ray)	0.003	0.2
Mr. H.	Aneurysm (Roentgen-ray)	0.05	0.2
Mr. F.	Aneurysm (Roentgen-ray)	0.025	0.2

In Protocol 6 it is seen that the two cases with slight dilatation of the aorta, although their serums differed in strength, required the same amount of antigen and a large amount of antigen to give complete inhibition. The case with a considerable diffuse dilatation required a little less antigen. The six cases with outspoken aneurysm whose serums differed in strength all required a still smaller amount of antigen, and the case which ruptured on the ward required even a little less than the others. Therefore, as the stage progressed from a slight dilatation through a moderate to a typical aneurysm and finally to rupture, the amount of antigen necessary to give complete fixation diminished, although the F. U. of serum bore no relation to the state of dilatation.

Observing from the foregoing protocols that the amount of serum necessary to give complete fixation was not constant for any given condition or stage of the disease, but that the amount of antigen necessary to give this complete inhibition was apparently quite constant, it was thought to be of interest to determine whether a definite increase in the amount of serum would make a correspondingly proportionate decrease in the amount of antigen required to give complete fixation. This was found to be the case, as shown in Protocol 7.

PROTOCOL 7.—Relation Between Amounts of Serum and of Antigen Required to Give Complete Fixation

Name	Stage of Disease	F. U. in c.c.	
		Serum	Antigen
Mrs. D.	Gumma under treatment	0.05 ++++	0.2 ++++
	.... with	0.01	0.1 ++++
Mr. S.	Aneurysm .... with	0.025 ++++	0.2 ++++
		0.1	0.05 ++++
Mr. D.	.... with	0.05 ++++	0.05 ++++
		0.1	0.025++++
Mr. N.	Aneurysm .... with	0.003 ++++	0.02 ++++
		0.012	0.05 ++++
		0.025	0.025++++
Mr. C.	Gumma .... with	0.025 ++++	0.05 ++++
		0.05	0.025++++

In this protocol five serums with a different F. U. were selected out of a large number to illustrate the fact that increasing the quantity of serum, proportionately decreased the amount of antigen necessary to give complete inhibition of hemolysis. In the first, third and fifth cases when the F. U. was doubled, the quantity of antigen was halved; in the fourth case the F. U. was quadrupled and the antigen quantity was quartered; in the second case the F. U. was first quadrupled and this quartered the antigen, again this amount was doubled and the amount of antigen required to give complete inhibition of hemolysis was halved. Therefore, a certain quantity of serum in each case required a definite quantity of antigen to give fixation, and there was a definite mathematical variation between antigen and serum. This was likewise true of spinal fluids. This held true in all cases up to a certain limit; that is, doubling and quadrupling the F. U. caused correspondingly diminishing amounts of antigen necessary. If the serum was sufficiently increased in the foregoing manner, however, a limit was reached at which the quantity of antigen did not decrease no matter how much serum was used.

The next step was to determine whether the preceding facts held for different strengths of the same antigen. The same serum was titrated for its F. U. with different strengths of the same antigen.

The cases of Protocol 8 were selected from a larger series, since they represent work done with four different heart antigens, each antigen being used in two or more definite strengths; that is, the 1 : 12 emulsion was one-half as strong as the 1 : 6 emulsion, and the 1 : 24 emulsion was one-fourth the strength of the 1 : 6 emulsion. It is evident that a definite variation in the strength of the antigen did not bear a corresponding definite variation to the quantity of serum necessary to give complete fixation. In other words, when a certain constant quantity of antigen gave a certain F. U. with a serum, a constant quantity of one-half the strength of antigen did not correspondingly increase the amount of serum necessary to give complete fixation. There was a wide indefinite variation in the quantity of serum necessary for its F. U. when the quantity of antigen was definitely varied.

PROTOCOL 8.—F. U. OF SAME SERUM WITH DIFFERENT STRENGTHS OF SAME ANTIGEN

Name	Stage of Disease	Antigen No.	Emulsion	F. U. of Serum c.c.++++
Mr. J.	Gumma of liver (autopsy)	1	1:6	0.003
		1	1:12	0.012
		6	1:6	0.003
		6	1:12	0.012
Mr. A.	Tertiary rash	6	1:6	0.05
		6	1:12	0.1
Mr. H.	Tabes	1	1:6	0.003
		1	1:12	0.012
Mr. J.	Tertiary	3	1:6	0.003
		3	1:12	0.05
		3	1:24	0.05
Mrs. M.	Latent	5	1:6	0.003
		5	1:12	0.003

In a number of cases the serum F. U. was determined with different definite strengths of antigen as in Protocol 8, and then each F. U. of serum was kept constant and titrated with diminishing amounts of antigens of different strength, and in addition the amount of serum was definitely increased and then titrated. A few of these titrations are given in Protocol 9.

The five cases in Protocol 9 were selected from a large series to illustrate the following points. As previously shown, in determining the F. U. of a serum the amount of serum necessary to give complete fixation with antigens of different strengths bore no definite relation to the quantity of antigen. That is, the quantity of serum, which was the F. U. with one antigen. did not always double in amount when it was the F. U. with an antigen of one-half the strength as might be expected; for instance, in the first case, Protocol 9, the F. U. of the serum was 0.05 c.c. with both strengths of antigen 1:6 and 1:12, and in the third case the F. U. of serum with 1:6 was 0.003 c.c., but with 1:12 it required four times the amount of serum, or 0.012 c.c. After the F. U. for the serum was determined, however, a definite increase in the quantity of serum caused a corresponding decrease in the quantity of antigen necessary to fix it. This point is illustrated in the first two cases in Protocol 9. Take the first case, for instance: 0.05 c.c. of serum required 0.05 c.c. of antigen of the strength 1:6 and twice the amount of serum, or 0.1 c.c., required one-half the amount of antigen 1:6, or 0.025 c.c.; also 0.05 c.c. of serum required 0.05 c.c. of antigen 1:6, but twice this amount or 0.1 c.c. of serum was necessary to give fixation with the same amount of antigen, 1:12, which is one-half as strong. Therefore, a definite increase in the amount of the serum caused the same definite decrease in the required amount of antigen and vice versa. The same facts were true where three different strengths of antigen were used as is shown in the last two cases of Protocol 9. When, however, as in the last two cases, this titration is carried far enough a limit is reached above which an increase in the

PROTOCOL 9.—Titration of Different Definite Strength of Antigens

Mr. D. serum F. U. with No. 6 (1:6 em.) 0.05 c.c. ++++
Serum F. U. with No. 6 (1:12 em.) 0.05 c.c. ++++
0.05 c.c. serum required 0.05 c.c. of antigen 1:6 and 0.1 c.c. antigen 1:12
0.1 c.c. serum required 0.025 c.c. of antigen 1:6 and 0.05 c.c. antigen 1:12

Mr. C. serum F. U. with No. 1 (1:6 em.) 0.025 c.c. ++++
Serum F. U. with No. 1 (1:12 em.) 0.05 c.c. ++++
0.025 c.c. serum required 0.05 c.c. antigen 1:6
0.05 c.c. serum required 0.025 c.c. antigen 1:6 and 0.05 c.c. antigen 1:12
0.1 c.c. serum required 0.1 c.c. antigen 1:12

Mr. N. serum F. U. with No. 1 (1:6 em.) 0.003 c.c. ++++
Serum F. U. with No. 1 (1:12 em.) 0.012 c.c. ++++
0.003 c.c. serum required 0.2 c.c. of antigen 1:6
0.012 c.c. serum required 0.05 c.c. of antigen 1:6 and 0.1 c.c. antigen 1:12
0.025 c.c. serum required 0.025 c.c. of antigen 1:6 and 0.05 c.c. antigen 1:12
0.05 c.c. serum required 0.025 c.c. of antigen 1:6 and 0.05 c.c. antigen 1:12

Mr. McM. serum F. U. with No. 6 (1:6 em.) 0.003 c.c. ++++
Serum F. U. with No. 6 (1:12 em.) 0.003 c.c. ++++
0.003 c.c. serum required 0.025 c.c. of antigen (1:6) 0.05 c.c. (1:12) 0.1 c.c. (1:24)
0.006 c.c. serum required 0.012 c.c. of antigen (1:6) 0.025 c.c. (1:12) 0.05 c.c. (1:24)
0.012 c.c. serum required 0.012 c.c. of antigen (1:6)

Mrs. S. serum with No. 7 (1:12 em.) 0.012 c.c. ++++
0.025 c.c. serum required 0.025 c.c. antigen (1:6) 0.05 c.c. (1:12) 0.1 c.c. (1:24)

serum causes no corresponding decrease in the quantity of antigen necessary to fix it. Therefore, a definite amount of serum required a definite amount of antigen up to a certain limit, but an excess of serum above this limit does not influence the quantity of antigen. For instance, in the last case 0.025 c.c. of serum required 0.025 c.c. of antigen 1 : 6, and twice this amount of serum or 0.05 c.c. required the same amount of antigen 1 : 6 and did not decrease the required amount of antigen, as was the case when smaller amounts of serum were used. This is probably explained by the fact that in the last instance we are using very large amounts of serum as compared to the F. U., and the quantity of antigen is very small; the quantity of antigen is approaching infinity and naturally sooner or later, a limit of value must be reached. Therefore, from the preceding statements one must conclude that there is a definite quantitative relation between serum and antigen.

Now, let us analyze this antigen in order to determine what accounts for this definite specificity. From the preliminary titrations of the alcoholic heart extract we know that it required 1.0 c.c. of a 1 : 6 emulsion to give fixation with a positive serum, but after the addition of 0.4 per cent. of cholesterin it required only 0.05 c.c. of 1 : 6 emulsion to give fixation with the same amount of a positive serum; therefore the cholesterin must play a most important part. Different alcoholic heart extracts containing cholesterin produce, when mixed with saline, different degrees of turbidity; one emulsion may be moderately turbid, whereas another emulsion may produce a very heavy suspension. One might consider that the difference in the turbidity of emulsions could account for variation in strength of an antigen. Such is not the case. For example, Antigen 7 formed a very slight turbidity when mixed with saline, Antigen 6 formed a heavy suspension and Antigen 4 formed a moderately heavy emulsion, still when the same serum was titrated against each antigen on the same day the serum F. U. was the same for each antigen. Furthermore, each antigen was centrifuged at high speed and the supernatant fluid was used as an antigen and the F. U. of serum remained the same as before centrifuging. Therefore, the heaviness of the emulsion or the particles forming the emulsion played no part in this specificity.

The only constituent remaining in this antigen which has not been ruled out is the cholesterin, and this is present in definite quantities in all emulsions. For instance, 0.05 c.c. of the 1 : 6 emulsion contains 0.000033 gm. of cholesterin, 0.05 c.c. of the 1 : 12 contains 0.0000165 gm. and 0.05 c.c. of the 1 : 24 contains 0.0000082 gm. of cholesterin. Referring, for example, to the third case, Mr. N. in the last protocol, it is seen that with the antigen 1 : 6 emulsion, 0.003 c.c. of serum was the F. U. When this F. U. of serum (0.003 c.c.) was titrated with diminishing amounts of antigen, 1 : 6, it required 0.2 c.c. of this antigen to give fixation, and this 0.2 c.c. of antigen contained 0.000132 gm. of cholesterin. When four times this amount of serum (0.012 c.c.) was titrated with the same 1 : 6 emulsion, only 0.05 c.c. or one-fourth the amount of antigen was necessary, this 0.05 c.c. represents 0.0000033 gm. of cholesterin. Eight times the amount of serum required only one-eighth the amount of cholesterin. The same facts hold true when the 1 : 12 emulsion was used. Furthermore, when the same amount of serum was titrated with two different strengths of antigen (0.025 c.c. of serum titrated with 1 : 6 and 1 : 12 emulsions), the same amount of cholesterin was necessary to give fixation, although the fluid quantity of antigen was twice as much in one case as in the other. Still further, when two portions of serum, the one twice the quantity of the other, were titrated with two different strengths of antigen, the larger quantity of serum being titrated with the weaker antigen and vice versa, the fluid quantity of antigen was twice as great in the case of the larger amount of serum with the weaker antigen, still the amount of cholesterin required in both was the same. The same facts also held true when spinal fluids were titrated in the same manner.

From the above-described methods in which this problem has been attacked in many ways — and in fact no other ways are apparent to me — it would seem quite conclusive that cholesterin played a most important part in the fixation test for syphilitic serums, and from these titration methods it would seem that a definite amount of cholesterin was necessary for the fixation of a definite amount of serum and varying the amount of serum likewise varied inversely the amount of cholesterin. The serum of syphilitic patients contains a "something" for which cholesterin has a definite fixation power in the presence of complement.

SUMMARY

The material for this paper consists of 1,000 Wassermann reactions performed on the blood of 800 different persons and 87 reactions performed on the spinal fluids of as many persons. The first few pages of this paper describe in detail the technic employed in the Wassermann reaction as used here; inasmuch as different workers use different technics, the results vary, and consequently a negative reaction is meaningless unless the technic and its general results are known. The next few pages are devoted to a discussion of the antigen. An alcoholic extract of human heart reinforced with 0.4 per cent. cholesterin was the antigen used, and for the reactions 1 part of this was added to 9 parts of saline, making a 1 : 10 emulsion; 0.5 c.c. of this emulsion was used in each test-tube. The advantages of such an antigen were: the ease with which it is made from any human heart; its constant antigenic properties; and especially its superior antigenic properties over other antigens with syphilitic serums, this superior sensitiveness being most evident in the late manifestations of syphilis.

No disadvantages are apparent when the antigen is carefully controlled and all reagents are accurately titrated. The remainder of the first part of the paper is taken up with the results obtained, using this as an antigen in the Wassermann reaction. Among the 800 persons, the serums of 667 patients gave a negative reaction, and in none of these was there a suspicion of syphilis. There were 110 patients whose serums gave a positive reaction, that is a complete inhibition of hemolysis, and there was no evidence of a false reaction. There were twenty-four cases with whose serums a partial fixation was obtained, and in all of these there was evidence of syphilis which had been modified by treatment, with the exception of one person with acute catarrhal jaundice, and when the jaundice disappeared, the reaction became negative. In a series of 400 cases, in addition to the 1:10 emulsion which was used for routine diagnosis, a 1:6 emulsion was used. This 1:6 emulsion is too near the anticomplementary dose for safety; still there were no false complete inhibitions of hemolysis, and in only 2 per cent. of the cases was there a false incomplete fixation. Furthermore, it was found that in the early chancre stage and in cases which had been treated, this 1:6 emulsion was of great value, and it was definitely noted that all cases should be treated until their serums became negative with this 1:6 emulsion if a relapse was to be avoided.

The second part of the paper deals with original work in methods of titration. It was found that the amount of serum necessary to give fixation varied greatly in different persons, and in different persons in the same stage of the disease. On the other hand, after the required amount of serum necessary to give fixation (the F. U.) was determined, the amount of antigen remained constant for different persons in the same stage of the disease. Furthermore, by this means it could be ascertained whether a given rash was an early or a late one; the same facts were true in cases with syphilitic disease of the aorta, an aneurysm required less antigen in amount than a slight dilatation of the aorta. Having found by titration the least amount of serum required to give fixation with the standard amount of antigen, and then determining the least amount of antigen required to give fixation with this least amount of serum, a definite increase in the amount of serum caused a proportionate definite decrease in the amount of antigen necessary to give fixation. By elimination experiments and by substituting the actual amount of cholesterin present in each portion of antigen used, it was found that the cholesterin was the specific agent which, in the presence of complement, combined with the syphilitic serums.

CONCLUSIONS

Cholesterinized alcoholic extracts of human heart have a specific fixation property with syphilitic serums, and they do not give nonspecific fixation with non-syphilitic serums, provided the reagents are accurately titrated.

The serum of syphilitic patients contains a "something" for which cholesterin has a definite fixation power in the presence of complement.

BOTULISM

A REPORT OF FOOD-POISONING APPARENTLY DUE TO EATING OF CANNED STRING BEANS, WITH PATHOLOGICAL REPORT OF A FATAL CASE *

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CLINICAL REPORT, BY DR. WILBUR

A meal of roast beef, string bean salad with mayonnaise dressing, gelatin pudding, coffee and whipped cream was partaken of by a group of young people at a sorority supper held at Stanford University on Nov. 25, 1913; twenty-four ate of the bean salad and of these twelve were taken ill within the succeeding few days with symptoms of botulism. The string beans were from two separate lots, one dark green in color, purchased at a grocery store and packed by a canning association; the others, light green in color, were put up the summer before at the home of one of the girls attending the dinner. These latter beans were apparently prepared by the process of sterilization recommended by the U. S. Department of Agriculture Bulletin, that is, boiling in the final container for one hour on three successive days. There was no food or meal taken in common by all of those poisoned except the one in question. Among those made ill was one of the waiters, who had likewise partaken of the beans. The fact that two or three of the first patients had also eaten mushrooms confused the diagnosis slightly at first. Of the twelve taken ill one died about two weeks after the meal. All of the rest had bulbar disturbances of varying degree but all made a satisfactory recovery.

The early symptoms came for the most part at the end of thirty-six to forty-eight hours and because of the eye manifestations several first consulted an eye specialist, Dr. Howard Black of Palo Alto, and he together with Dr. Thomas M. Williams were in attendance on the patients whom I had the opportunity of studying. While there was marked variability in the course and seriousness of the disease picture all were enough alike so that the description of one patient will illustrate the phenomena observed.

Girl, aged 23, Tuesday evening, Nov. 23, 1913, ate the dinner including the canned string beans of the light green color together with a little rare roast beef. The following day she felt perfectly normal except that at 10:00 in the evening the eyes felt strained after some sewing. Thursday morning,

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thirty-six hours after the meal, when the patient awoke, the eyes were out of focus, appetite was not good and she felt very tired. At night she had still no appetite, was nauseated and vomited the noon meal apparently undigested. Friday morning, two and one-half days after the meal, the eyes were worse, objects being seen double on quick movement, and it was noticed that they had a tendency to be crossed. A peculiar mistiness of vision was also complained of. She was in bed until late in the afternoon, when she visited Dr. Black. She had had some disturbance in swallowing previous to this time and stated that it felt as if "something came up from below" that interfered with deglutition. The fourth day she remained in bed, was much constipated, and noticed a marked decrease in the amount of urine voided. There was at no time pain except for occasional mild abdominal cramps, no headache, subnormal temperature, and a normal pulse. The fourth and fifth days the breathing became difficult at times and swallowing was almost impossible. The patient complained of a dry throat with annoying thirst. The sixth day there were periods of a sense of suffocation with a vague feeling of unrest and as if there might be difficulty in getting the next breath. The upper lids had begun to droop. The voice was nasal. When the attempt was made to swallow liquids they passed back through the nose. The patient felt markedly weak.

Physical examination at this time showed ptosis of both upper eyelids, dilatation of the right pupil, sluggish reaction to light of both pupils, apparent paralysis of the internal rectus of the left eye, normal retina, inability to raise the head, control apparently having been lost of the muscles of the neck, inability to swallow, absence of taste. The tongue was heavily coated and the throat was covered with a viscid whitish mucus clinging to the mucous membrane. The soft palate could be raised but was sluggish, particularly on the right side. The exudate on the right tonsil was so marked that it resembled somewhat a diphtheritic membrane. The seventh day there was some change in the condition; occasional periods occurred when swallowing was more effective, and there was less tendency to strangle. On the eleventh day there was some improvement of the eyes, still strangling on swallowing, sensation of taste was keener, and the general condition improved. The twelfth day the patient was able to move her head, but was unable to lift it except when she took hold of the braids of her hair and pulled the head forward. The eyes could be opened slightly, speech was less nasal and more distinct, and improvement in swallowing was marked. At the end of two weeks the patient was able to take soft diet freely, and at four weeks she was up in a chair for a couple of hours complaining only of general weakness and inability to use her eyes. At the end of five weeks she was able to leave the hospital and return to her home and later to resume her regular work.

The foregoing description is typical of the cases seen and corresponds to the picture of botulism, which was first clearly differentiated as a clinical entity by Van Ermengen, who, following his study of the epidemic of Ellezelles, demonstrated that the symptoms were due to a toxin formed by an anaerobic organism, the *Bacillus botulinus*. In the patients seen by me there was a wide variation in the severity of the disease, some having only transient disturbance of vision or of swallowing, others such complaints as "jaws seemed very tired," "apparent inability to chew food," "tongue seemed hard to move," "sleepy all the time," "could not walk fast," "throat filled up with mucus all the time," "tried to attend classes, legs and arms were almost powerless," "hard work climbing stairs," and "throat felt as if there was shelf in it beyond which food could not pass but it did not hurt." The complaint of seeing double and of the mist passing over the eyes was comparatively common. The gradual onset of the symptoms and their marked variability were the most striking features. At one time a patient would show marked inability to swallow, while a little later a soft meal would be taken without difficulty. There was very little difference for some time in the severity of the clinical picture between the fatal case and some of the others. The details of these cases were reported by Dr. Thomas M. Williams at the meeting of the California State Medical Society in April, 1914.

FATAL CASE

Young healthy woman, aged 23, on the second morning following the ingestion of the meal at which time the beans were taken, complained that objects were often seen double. There was no headache or vomiting; urination apparently as usual. A laxative had been taken because of constipation and the bowels had acted. On the fourth morning the patient was unable to eat because of the inability to swallow. There was no pain. Later on in the day she swallowed with difficulty. On the sixth morning her mouth was dry, there was considerable thirst, no abdominal colic, no appetite, marked asthenia, with the muscular power in the left arm less than normal. Voice was slightly nasal with inability to articulate distinctly. Temperature was 97.8 to 99 F., pulse 104. There was ptosis of both upper lids; both pupils were dilated but reacted to light, with normal fundi. The breath was foul, tongue coated and it and the pharynx covered with sticky, whitish, viscid mucus. There was considerable edema of the uvula.

The condition did not change very much in the next few days except that the patient felt very weak, was unable to raise the head without help, at times would swallow well, at other times was unable to take the simplest food, and the pulse became more rapid and dicrotic. Blood-pressure was normal. At one time she became quite talkative and complained that her jaws did not open naturally and she could not see well but for the most part she was quiet and slightly depressed.

On the tenth day she brought up with very much difficulty some mucus which had been interfering greatly with her throat and had a period in the afternoon when she was unable to swallow, had difficulty in breathing and cold extremities. Later on she was able to take an egg-nog, but complained of a spontaneous choking sensation. In the night to the distress from these attacks was added an inability to breathe through the nose.

On the twelfth day involuntary movements of the bowels occurred. Some relief from the choking, breathless sensation was gained by swabbing the throat. The patient vomited some dark green fluid after a large, soft involuntary stool and began to feel very warm and excited, axillary temperature going up to 100.4. After a period of chills, great restlessness, choking sensation, increasing temperature and higher pulse rate together with the signs of hypostatic pneumonia, the patient had a collapse from which she recovered on the administration by Dr. Williams of an intravenous transfusion of normal salt solution. She vomited later a considerable amount of blood and although her breathing became more superficial, her weakness more evident, the sensorium remained perfectly intact.

On the thirteenth day she died following a respiratory disturbance apparently due to sudden paralysis of the diaphragm, since the heart continued to beat for some time. Various form of stimulant and other treatment were administered without avail. The necropsy, performed by Dr. William Ophüls, was practically negative except for areas of collapse and bronchopneumonia in the posterior parts of the lungs, and certain lesions of the central nervous system, which will be described later.

SYMPTOMS AND SIGNS

When one sees a series of cases presenting such striking features as those enumerated in the two described the difficulty of making a diagnosis of botulism is not great, but it is easy to see how a sporadic case could be readily overlooked or confused with various other conditions, such as luetic bulbar paralysis, anterior poliomyelitis, etc. The symptoms noted by the various writers who have described epidemics of this type are all of the same general character.

DIGESTIVE AND SECRETORY SYSTEMS

Contrary to the usual idea of food-poisoning, there is apt to be freedom from pain except perhaps for some colic, occasional vomiting, and only at times diarrhea, in patients who have been poisoned with botulismus toxin. Obstinate constipation is the rule, the intestinal tract being paralyzed by the ingested poison. Bürger[1] reports the washing out from the stomach of food particles eaten five days previously, on the evening of the day when the infected ham was taken for breakfast. Slow absorption from this alimentary tract paralysis probably explains in part the gradual onset of the symptoms and also the finding by Kob of toxin in the blood of a child eight days after the ingestion of the infected food. Very little peristalsis was to be heard over patients seen by me except after the use of brisk cathartics and enemas. The late involuntary stools and the other gastro-intestinal features in some cases may be due to a central effect on the vagus. The first stool passed by a patient was described by Van Ermengen[2] as dark and full of mucus. Anorexia is common, and the foul breath, coated tongue, viscid, sticky, whitish mucous secretion with the generally dry mouth is accompanied apparently by a paralysis of the secretory activity of many glands (parotid enlarged at times) extending in some patients even to the lacrimal glands, so that Schumacher[3] reports the inability of a woman to shed tears when told of her husband's death. At times tears are secreted freely, and, as in one of our patients, the eyes appear watery and injected.

NERVOUS SYSTEM

The most striking effect on the nervous system is the difficulty in vision with the normal background, dilated pupils, ptosis, and paralysis of the various eye muscles with double vision and the disturbance in deglutition. Dizziness, sleeplessness, marked muscular weakness with no disturbance of the sensorium, no headache and no anesthesia or paresthesia are characteristic. At times various paralyses of the different skeletal muscles particularly about the neck are seen. Practically all the cranial nerves except the first and second have shown involvement in described cases; that is, ptosis, mydriasis, disturbances of accommodation (third and fourth cranial nerves), inability to cry (lacrimal nerve of the fifth cranial), dryness in the mouth (seventh and ninth cranial), disturbance of deglutition partially due to lessened secretion and partially due to nervous disturbances leading to difficulty in chewing and the muscular side of swallowing (ninth, tenth and eleventh cranial nerves), mask-like expression (seventh cranial), at times difficulty in hearing (eighth cranial), variation in speech to aphonia (twelfth), obstipation and respiratory and cardiac disturbances (tenth cranial nerve). The retention of the urine and the occasional paralyses of muscles of the limbs indicate that the spinal cord may be damaged at times. The deep reflexes are not disturbed. Romberg's and Babinski's signs both absent. No tremor and no spastic conditions have been described. We noted no angioneuroses of the skin. The sensations of the mucous membrane of the throat and mouth are dulled at times probably because of its dry condition.

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[1] Bürger: Ueber zwei Gruppen von Botulismus mit zwölf Erkrankung- und fünf Todesfällen, Med. Klin., 1913, ix, 1846.

[2] Van Ermengen: Ztschr. f. Hyg. u. Infectionskrankh., 1897, xxvi, 1.

[3] Schumacher: München. med. Wchnschr., 1913, lx, 124.

CIRCULATORY AND RESPIRATORY DISTURBANCES

The temperature, pulse and respiration remain practically normal unless the vagus center is involved or a secondary infection takes place. The blood-pressure was found normal in our patients, and no abnormalities were noted in the blood itself. The feeling of compression and oppression in the chest complained of by some patients is probably nervous, not circulatory, in origin.

GENITO-URINARY SYSTEM

A lessening in the amount of practically normal urine is usually seen. At times this is due to retention. There may be paralysis of the bladder detrusors or sphincters. The small amount of urine later is perhaps due to the inability to swallow. Twenty-four hours or longer passes in some patients before urine is voided.

Throughout it must be constantly remembered that the symptoms may be extremely acute and followed by early death, or they may be gradually unfolded in their entirety, or only one or two characteristic evidences may appear.

The variations in the course of the disease may be due to the amount of toxin in the food eaten, to individual resistance or to the amount of vascular thrombosis present. The prognosis for eventual complete recovery is good if the early part of the illness is survived. In the cases here reported only one of the twelve patients succumbed, although no early difference in the severity of the clinical picture could be seen between this patient and some of the others who recovered. Death usually occurs during the first or second week, seldom later. From 30 to 50 per cent. is a common death-rate. In the Ellezelles epidemic described by Van Ermengen only three of the thirty patients died.

DIFFERENTIAL DIAGNOSIS

The isolated case is the one that is most apt to lead to confusion in diagnosis, particularly with syphilis and poliomyelitis. When botulismus is severe enough to endanger life promptly, the disease picture is usually so distinct as to permit of ready recognition. The prominence of the paralysis of the eye muscles often brings the patient first to the ophthalmologist, who must bear in mind that conditions varying from a nystagmus to an absolute immovability of the eyeballs, always with a normal retina, may occur in botulismus. Poliomyelitis, the so-called asthenic bulbar paralysis, cerebral syphilis, the various toxic ophthalmoplegias, rabies, diphtheria, poisoning with plant alkaloids (atropin, gelsemium, hyoscyamin, etc.), methyl alcohol, and ptomains (methylguanidin, ethylendiamin, etc.) must all be ruled out before making a final diagnosis.

ANATOMICAL LESIONS, BY DR. OPHÜLS

As a result of van Ermengen's classical work the question of the pathology of botulismus appears to be well settled. The bacteriological findings have been confirmed by several later investigators and his animal experiments hardly leave any doubt as to the etiology of the disease. Although the symptoms produced in the experimental animals by means of administration of the toxin of the *Bacillus botulinus* are perhaps not absolutely identical with those observed in human beings, they are similar enough to recall the symptom-complex of the disease in man and moreover they coincide entirely with the result of intoxication of similar animals treated directly with the spoiled foodstuffs.

On the basis of the *clinical symptoms* largely one has assumed a specific action of the botulismus toxin on the ganglion cells of the central nervous system, although other effects of the poison have been observed, namely, a marked paralyzing effect on the gastro-intestinal canal and a tendency to the occurrence of multiple minute hemorrhages, more especially in pons and medulla. The former, the paralysis of stomach and bowel, might of course also be ascribed to an affection of the motor ganglia of these organs, either centrally or peripherally.

This conception of a specific action of the toxin on the ganglion cells has been much strengthened by the histological findings in the central nervous system of animals subjected to the experimental intoxication. Van Ermengen in his original article[4] describes peculiar degenerative lesions in the ganglion cells of such animals which involve largely the Nissl granules in them, although the nucleus also appears sometimes affected. Of these changes he says:

> "There are almost none in the cerebrum or in the cranial nerves, but they are very pronounced in the spinal cord, less so in medulla, in pons and in the peduncles." He adds, however: "On the whole these lesions are very variable in intensity."[5]

He also finds small perivascular hemorrhages. Marinesco,[6] who studied the same material, gives practically the same account of the alterations. He even speaks of a phagocytosis of the injured cells by neuroglial phagocytes. Kempner and Pollack[7] confirm these observations of Marinesco and of van Ermengen, but believe that in their specimens, also obtained from animals treated with the botulismus toxin, they observed a still earlier lesion of the Nissl granules, which they speak of as *klumpige Schwellung.* They did not find any evidence of phagocytosis. The question was reexamined experimentally by Ossipoff[8] in 1900. He experimented on monkeys, cats and pigs as the animals most susceptible and found lesions in the nerve-cells similar to those described by Marinesco and others. He also believes to have found evidences of phagocytosis of the damaged cells. He makes the following interesting statement:

> "Within the hyperemic blood-vessels as well as on the outside of them there were found many white blood-corpuscles. In the vessels these corpuscles were sometimes bunched in masses."[9]

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[4] Van Ermengen: Contribution á l'étude des intoxications alimentaires, Arch. de Pharmacodyn., 1897, iii, 213, 499.

[5] "Elles sont presque nulles dans le cerveau, absentes dans les nerfs craniens et très accusés dans la moelle épinière, moins prononcées dans le bulbe, la protuberance et les pedoncles." "Ces lésions, considérées dans leur ensemble, sont très variable comme intensité."

[6] Marinesco: Pathologie générale de la cellule nerveuse. Lésions sécondaires et primitives. IV. L'action du bacillus botulinus, La Presse Méd., 1897, v, 43.

[7] Kempner and Pollack: Die Wirkung des Botulismustoxins (Fleischgiftes) und seines specifischen Antitoxins auf die Nervenzellen, Deutsch. med. Wchnschr., 1897, xxiii, 505.

[8] Ossipoff: Influence de l'intoxication botulinique sur le système nerveux central, Ann. de l'Inst. Pasteur, 1900, xiv, 769.

[9] "Dans les vaisseaux hyperemiés ainsi qu'en dehors d'eux on rencontrait aussi beaucoup de globules blancs. Dans les vaisseaux il y avait parfois une agglomération de ces globules."

The importance of this statement will become more evident in the discussion of our own findings. On the basis of the findings in the ganglion cells, Römer and Stein[10] attempted to localize more exactly the paresis of accommodation observed in some of the animals experimented on and came to the conclusion that the disturbance is localized in the nucleus of the oculomotor nerve, on account of the degenerative lesions found in certain parts of the nucleus.

It is surprising how few human brains have been the object of a careful histological investigation in this disease. Van Ermengen[4] was unable to obtain any satisfactory material in his cases. Fischer,[11] who describes an outbreak of the disease due to spoiled beans, clinically came to the conclusion that the intoxication produced "central paralysis with successive elimination of certain motor nuclei in the brain stem and of the vital centers in the floor of the fourth ventricle," also that it eventually caused a descending paralysis of the motor neurons in the anterior gray columns of the spinal cord. Parts of the brains in several of his cases were sent to the Pathological Institute at Giessen for examination, but apparently no characteristic lesions were found. In the latest publication by Bürger several new post-mortem examinations on cases dead of botulism are mentioned but in *one* of the records only there is this short allusion to specific lesions in the central nervous system:

> In the cerebrum the ganglion cells are well preserved, but in the region of the oculomotorius they show commonly dust-like disintegration of the Nissl's granules and eccentric position of the nuclei.[12]

It is well to bear in mind in this connection how equivocal these finer lesions in the bodies of the ganglion cells still are. So far as I can see they are given less significance at the present time by neurologists than they were shortly after their discovery by Nissl.

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[10] Römer and Stein: Experimenteller Beitrag zur Frage nach dem Sitz und Wesen der Accommodations-parese bei bakteriellen Intoxikations-Krankheiten, Arch. für Ophth. (Graefe's), 1904, lviii, 291. "Die im Verlaufe der Botulismus-Vergiftung auftretende Accommodations-parese hat ihren Sitz im Oculomotorius-Kerngebiet und ist eine durch bestimmte Komponenten des Botulismus-Toxins hervorgerufene Degeneration von Ganglienzellen im Oculomotorius-Kern."

[11] Fischer: Ueber eine Massenerkrankung an Botulismus infolge Genusses verdorbener Bohnenkonserven, Ztschr. f. klin. Med., 1906, lix, 58. "Wir gewannen ferner aus den klinischen Beobachtungen den Eindruck dass es sich bei der Giftwirkung um das Auftreten zentraler Lähmungen handelt, um fortschreitende Ausschaltung der Kerne bestimmter motorischer Gehirnnerven im Gehirnstamme und der lebenswichtige Vorgänge auslösenden, automatischen Zentren im Boden der Rautengrube." "dass das Toxin vom Gehirnstamme und der Medulla allmählich absteigend die dem motorischen Apparate zugehörenden Neuronen der Vorderhörner des Rückenmarkes lähmt."

[12] Bürger: "Im Grosshirn waren die Ganglienzellen gut erhalten, während sie im Oculomotoriusgebiete vielfach staubförmigen Zerfall der Nissl's Granula, excentrische Lage des Kernes usw. erkennen liessen," Med. Klin., 1913, ix, 1846.

The brain in our case was obtained within twenty-four hours in a very good state of preservation. It has been remarked by others that in botulism the tissues remain intact for an unusually long period of time, which might suggest some interference with the normal autolytic ferments.

The gross findings at necropsy were entirely negative except for a moderate hyperemia of the cerebral vessels, which was especially pronounced at the base of the brain and in the region of the fourth ventricle, where there also was found some edema. No hemorrhages could be made out with the naked eye on several sections through the brain stem. The internal organs were markedly hyperemic, which was especially noticeable in liver, kidneys and intestines. This general hyperemia has been a constant finding in all cases of human botulism and is also found in the experimental disease in animals. The dilatation of all blood-vessels was also very evident in sections from all organs examined. Typical bronchopneumonic lesions were found in the lungs, which condition probably accelerated the death of the patient.

The following is a short description of the microscopical findings in the brain:

The brain stem was sectioned transversely at different levels at the time of the autopsy and carefully hardened in frequently changed absolute alcohol. Numerous sections were made from all levels and special attention paid to the regions of the more important nuclei. Sections were obtained from all motor and some of the sensory nuclei on both sides. The nuclei studied were those of the oculomotorius (third), trochlearis (fourth), trigeminus (fifth), abducens (sixth), facialis (seventh), acusticus (eighth), glossopharyngeus (ninth), vagus (tenth), and hypoglossus (twelfth) nerves. The following lesions were found:

Thrombosis of the right arteria vertebralis at lower end of the medulla and extending into the arteria basilaris some distance. The middle part of the arteria basilaris is free. The anterior portion of it again is filled with thrombus. There are thrombi in a few of the pial branches of the basilar artery. Many pial veins at the base of the medulla and pons are filled with thrombi more especially on the right side and there are several thrombi in the veins of the tela chorioidea above the corpora quadrigemina and the posterior part of the third ventricle. All blood-vessels in the brain-tissue are much distended and full of blood. Both in the ventral and in the dorsal parts of the brain-stem one finds quite a few small thrombosed blood-vessels, some of which are situated near important nuclei; for instance, one such was found near the right vagus nucleus, several in the nuclei of the olives, several in the substantia ferruginea, several in the right nucleus trochlearis, several near the right nucleus of the oculomotorius and similarly several near the left nucleus oculomotorius, but somewhat further away from it. Small perivascular hemorrhages were encountered quite commonly about thrombosed veins and others. The thrombi in all places were very rich in polymorphonuclear leukocytes, but no bacteria could be demonstrated in spite of the use of various staining methods, including Weigert's modification of Gram's method. All thrombi were very rich in fibrin and contained comparatively few masses of conglutinated blood platelets.

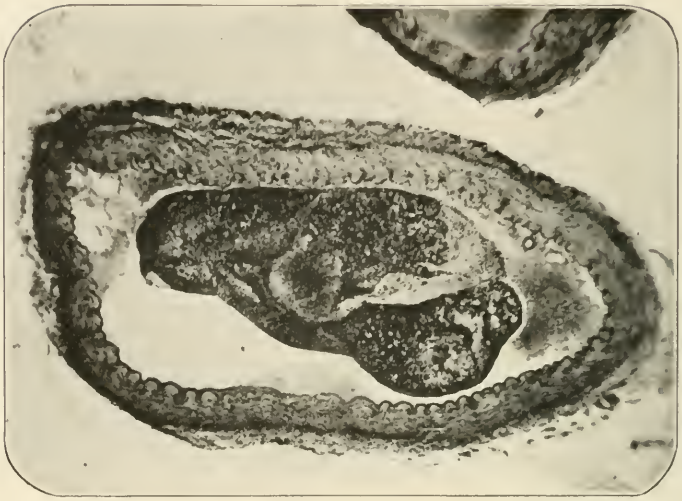

Fig. 1.—Thrombus in vertebral artery (from photomicrograph).

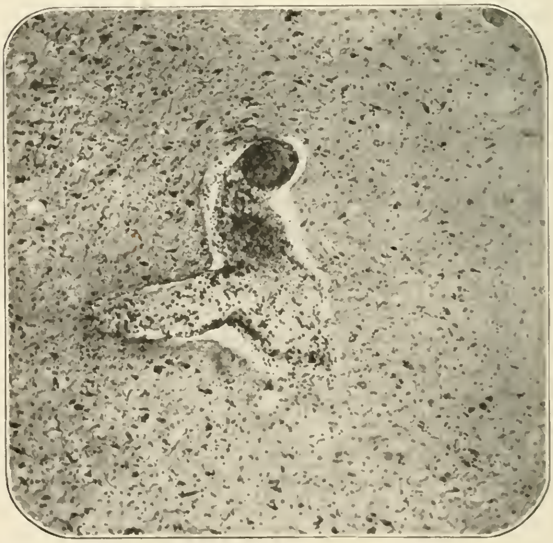

Fig. 2.—Thrombosis in small vein in brain tissue near oculomotor nucleus (from photomicrograph).

The Nissl granules stain unusually well in all sections especially in the large motor ganglion cells. They are quite normal in size and arrangement. Nothing unusual could be detected in spite of especially careful study of them. The nuclei of the ganglion cells are perfectly normal.

Sections were also taken from various parts of the cortex of the cerebellum. There was no difference in the appearance of the ganglion cells from these other regions. Few thrombi were seen in the pial veins. The blood-vessels in the cortex are dilated and full of blood, but none show thrombosis or perivascular hemorrhages.

No thrombi were found in any other organs in spite of the study of many sections except one in a vein in the submucosa of the intestine and the very beginning of the thrombotic process in few of the dilated vessels in the ovarian cortex.

Anaerobic cultures were made from the intestinal content both of the small and large intestines, from the cerebrospinal fluid, from the heart blood, and from the spleen. The cerebrospinal fluid and the heart blood were sterile and the *Bacillus botulinus* was not found in any of the other cultures.

I believe that on the basis of these findings it will be necessary to change considerably our ideas in regard to the action of the botulinus toxin. No evidence was found to substantiate the hypothesis of a specific action of the toxin on the nerve-cells. The negative findings in regard to any disturbance in the Nissl granules in our case are quite convincing in this regard in spite of the evidence to the contrary brought forward by other writers. The statement of Bürger[12] is so short and the lesions described in it are so slight that it hardly deserves much serious attention. But even so the fact remains that in our case the size and arrangement of the Nissl granules was quite perfect and if there should have been minor lesions they certainly were not sufficient to account for the severe clinical symptoms. So far as the positive findings in the experimental animals is concerned it may be pointed out that in them the localization of the lesions as described by Marinesco[6] and van Ermengen[4] and others does not coincide altogether with the clinical symptoms. They both emphasize the fact that the most marked involvement is found in the ganglion cells, both motor and sensory of the cord, whereas, in man at least, cord symptoms are late and rare and sensory symptoms have never been observed. The lack of histological findings in the ganglion cells does not of course prove that the poison may not have a specific action on the ganglion cells after all, but the matter remains a mere hypothesis which is greatly weakened by the positive finding of lesions which explain the clinical symptoms as well, if not more adequately.

There is present all evidence of a serious interference with the circulation in the meninges and in the brain which is most marked at the base of the brain, throughout the brain-stem where numerous hemorrhages testify to the seriousness of circulatory disturbance. Associated with this disturbance of the circulation one finds multiple thromboses in the blood-vessels, in both the arteries and the veins. The upper part of the right vertebral artery, the posterior part of the basilar artery and again the anterior part of the same vessel are completely obstructed by thrombi. Several of the branches of these arteries in the pia are filled with thrombi. There are thrombi in many veins in the pia and in the substance of the brain, several within or in the immediate vicinity of important nuclei.

The disturbance in the blood-vessels evidently consists in an extreme vasodilatation with great slowing of the blood-current, and as such it is only part and parcel of a general paralysis of the vasculatory mechanism which has been observed and noted by all careful observers of cases of botulism. This has been usually referred to insufficient heart action. When we take into account, however, that the poison has a powerful paralyzing effect on the involuntary muscles of the gastro-intestinal tract, as is shown by the prompt paralysis of the stomach and the obstinate constipation, it would appear probable that the poison might have a similar effect on the muscular wall of the blood-vessels, either directly or indirectly through the vasomotor nerves, which would certainly have a tendency at least to contribute greatly to any interference with the circulation which, however, might be partly due to poor heart action.

Why the circulation in all cases should be especially poor at the base of the brain, more especially in pons and medulla, is not so evident. One might suggest, however, that the main blood supply to these parts through the vertebral arteries is not so abundant as the blood-supply to the cerebrum from the internal carotid arteries.

The origin of the thromboses is also somewhat puzzling. I believe the existence of them in both arteries and veins excludes the idea of embolism.

It is also somewhat difficult to explain why we should have in these cases a tendency to thrombosis so exclusively in the blood-vessels of the brain, because in other organs very little evidence of thrombosis could be detected, but even if we assumed a pretty general tendency to thrombosis those in the blood-vessels in the brain stem would provoke the most prominent symptoms on account of the crowding of so many important centers at that point.

At all events in our case the evidences of severe disturbance of circulation associated with hemorrhages and thrombosis in pons and medulla are clear and they are sufficient to explain the symptoms, whereas the assumption of specific action of the poison on certain ganglion cells, in our case, appears to be purely hypothetical.

TREATMENT

As soon as the possibility of botulism presents itself the most evident therapeutic procedure is to empty the stomach thoroughly with a stomach tube, to wash out the large bowel and to leave in the stomach a brisk cathartic—castor oil or Epsom salts. Since the toxin is the cause of the symptoms and its absorption is slow because of the paralysis of the intestinal tract the foregoing measures should be carried out even after several days. The patient should be kept absolutely quiet, the body supplied with plenty of water, by rectum or under the skin if necessary, simple food given and great care used to prevent aspiration pneumonia. Strychnin seemed beneficial in the cases described in improving the action of the disturbed nervous centers. Other stimulants should be given as required.

The vascular conditions above described indicate the possibility of complete recovery from a condition that seems hopeless and should lend encouragement to keeping up a hard fight against the disease, even to prolonged artificial respiration in case of respiratory failure. The specific serum treatment has not been used in this country so far as I know. Leuchs[13] and others have prepared a serum which is very effective in animals. Polyvalent serums have given the best results. In Berlin this serum is prepared by the government. It is quite evident from the animal experiments that it would have to be near at hand and used promptly to be successful. That such serums should be kept in the various centers of the country is evident, as extensive botulism poisoning is apt to occur at any time.

SOURCE OF POISON

In attempting to determine the foodstuff responsible for the poisoning of the described patients each one was carefully questioned as to foods partaken of at the meal in question. As noted, there were two kinds of string beans, one dark in color from a grocery and put up by a promient canners' association, and another light green in color, domestically prepared during the previous summer. These latter beans were tender and were served as salad without preliminary heating by the Japanese cook. With mayonnaise dressing they tasted well and were eaten freely by those to whom they were served. Some of the guests were given only the dark-colored beans. None of these were taken ill. The symptoms of poisoning were so delayed that none of the bean salad was obtainable for examination but two extra cans of the same lot of home canned beans were placed at our disposal. The cans of tin were of quart size and the top was sealed with the hard red sealing wax often used for fruit jars, etc. One of these contained perfectly normal-appearing beans in a small amount of juice; the other was "blown" somewhat and its contents fermented and slightly sour. Juice from both cans injected into animals had no evident toxic effects. In the fermented can Dr. R. H. Major found a long spore-bearing bacillus resembling somewhat the *Bacillus botulinus.* It was not that organism, but an unidentified one, apparently the so-called "bacillus Tourné," well known and dreaded by fruit- and catsup-canners because of the resistance of its spores to heat and its ability to spoil a pack when mixed with the raw product. Evidently the spores of the above-mentioned organism withstood the fractional sterilization of boiling of one hour on three successive days; so, while we did not find the *Bacillus botulinus* in the beans, we did find an organism which grows under similar conditions. The possibility then was present that had the beans been contaminated with *Bacillus botulinus,* that organism could have developed in one of the cans. Therefore the question of whether the string beans may provide a suitable medium is of the greatest interest in view of the present cases. The resistance of the spores of *Bacillus botulinus* to heat, (while less than that of many anaerobes), the favorable anaerobic conditions, the optimum growth at ordinary temperatures, the ability to grow either in faintly alkaline or acid mediums all favor the view that this is possible.

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[13] Leuchs, J.: Ztschr. f. Hyg., 1910, lxv, 55; Kolle and Wassermann: Bacillus Botulinus (Immunität), Handbuch der Pathogenen Mikroorganismen, 1912, iv, 939 (full bibliography given).

The famous Darmstadt epidemic was due to eating preserved bean salad served without heating. These were apparently ordinary beans (wax rather than string beans) prepared in a cooking school (Fischer[14]). Landmann[15] suggests the possibility that small contaminated meat particles were mixed with the beans but brings out no evidence in support of this idea. The characteristic butyric acid (rancid) odor and slightly sour taste makes beans used for salad particularly dangerous, since with the usual dressing a slightly sour taste is agreeable to most individuals. The destruction of the toxin by heat was well illustrated in Darmstadt by the man who came late to the celebration and thinking the beans were being served as a vegetable and not as a salad, heated them along with other food and suffered no consequences. That canned green beans may also serve as a medium for the growth of other organisms is shown by the epidemic in Leipsic in 1906 described by Rolly,[16] in which the *Bacillus coli* and *Bacillus paratyphosus B.* were later isolated from them. In California several instances of poisoning resembling botulismus have come to our attention. Sheppard[17] describes in a graphic manner the typical and fatal poisoning of two men and a woman while on a camping trip following the eating of canned pork and beans. Peck[18] reported a similar and more extensive poisoning with eleven deaths at Sawtelle, Cal., in which he thought that canned pears were responsible.

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[14] Fischer, August: Ueber eine Massenerkrankung an Botulismus infolge Genusses "verdorbener" Bohnenkonserven (Nach Vorträgen, gehalten im ärztlichen—8/2/04—und im naturwissenschaftlichen Verein—8/3/04—in Darmstadt), Ztschr. f. klin. Med., 1906, lix, 58.

[15] Landmann, G.: Ueber die Ursache der Darmstädter Bohnenvergiftung, Hyg. Rundschau, 1904, xiv, 449.

[16] Rolly: Ueber Eine Massenvergiftungsepidemie mit Bohnengemüse (Bact. coli und Bact. paratyphi B), München. med. Wchnschr., 1906, liii, 1798.

In order to study the canned goods on which the *Bacillus botulinus* would grow, with the assistance of Drs. E. C. Dickson and E. G. Gary, a series of experiments were begun with a culture of *Bacillus botulinus* obtained from the New York Museum of Natural History by inoculating a number of cans of beans and keeping them under various conditions at various temperatures. Up to date one can tested had a slightly pungent smell and rabbits inoculated with the juice died with symptoms suggestive of botulism. The experiments will not be complete for some months, but they apparently point to string beans in sealed cans as a medium on which the *Bacillus botulinus* will grow. Further we cannot go at present. Van Ermengen[19] has carefully worked out the details of the growth of this organism, its toxin and its destruction under varying conditions, and they need not be reviewed here. The only record found of this organism occurring in nature was that of Kempner and Pollack, who found it in pigs' feces. We have as yet been unable to find out whether pigs were kept near the beans in question or whether the beans were fertilized with pigs' feces. It is fortunate that an organism with such possibilities for harm is rare.

Nevertheless the greatest care should be used in the preparation of all canned foodstuffs, particularly those kept under anaerobic conditions and apt to be eaten uncooked, to see that sufficient steam pressure and heat is used to kill all organisms and their spores. The sterilization under high pressure in vogue with all large canning companies makes their pack safe, but with the variability in domestic preparation the least rancid odor should lead to rejection of canned food and all vegetables should be reheated before being served. A long series of experiments on the penetration of heat into the interior of cans in preserving meats, etc., have been made by Bischoff and Wintgen,[20] Pfuhl,[21] Wolffhügel and Hueppe[22] and others and the need of steam pressure as well as heat is emphasized. Bolduan[23] describes the work of Belser and others on "blown cans" and found that a number of different bacteria more or less resistant to heat were present.

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[17] Sheppard: Report of Three Cases of Fatal Ptomain Poisoning, South. California Pract., 1907, xxii, 370.

[18] Peck, G. W.: South. California Pract., 1910, xxv, 121.

[19] Van Ermengen: Der Bacillus Botulinus und der Botulismus, Handbuch der Pathogenen Mikroorganismen (Kolle und Wassermann) 1912, iv, 909 (with full bibliography).

[20] Bischoff, H., and Wintgen, M.: Beiträge zuh Conservenfabrikation, Ztschr. f. Hyg., 1900, xxxiv, 496.

[21] Pfuhl, E.: Beitrag zur bakteriologischen Untersuchung der Fleischvonserven, Ztschr. f. Hyg., 1904, xlviii, 121.

[22] Wolffhügel and Hueppe: Mitt. a. d. k. Gsndhsamte, 1881, i, 395.

The possible relationship of epidemics to *Kalbfieber* (milk sickness) because of its similarity to botulism must be borne in mind (Crawford,[24] Schneidemühl[25]).

CONCLUSIONS

1. The twelve cases reported presented the typical clinical picture of botulism apparently due to eating of domestically prepared canned string beans served without preliminary heating. That string beans may act as a culture medium for the *Bacillus botulinus* is possible, and the possibility is being further investigated. The methods of sterilization under pressure in use by canning factories seems to preclude the possibility of the growth of *Bacillus botulinus* in their pack.

2. Vascular thromboses were found about the brain-stem in a patient dying with the symptoms of botulism. Such lesions explain better the clinical phenomena observed than does the more or less hypothetical assumption of a specific action of the botulin on certain ganglion cells.

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[23] Bolduan: Food Poisoning, Treat & Co., 1909, p. 104.

[24] Crawford, A. C.: The Supposed Relationship of White Snake Root to Milk Sickness or "Trembles," U. S. Dept. Agric., Bureau Plant Industry, Bull. 121, 1908, p. 5.

[25] Schneidemühl: Ueber Botulismus beim Menschen und die sogenannte Geburtsparalyse bei Rindern, Centralbl. f. Bakteriol., 1898, xxiv, 577.

A NOTE ON THE NORMAL PECULIARITIES OF THE HEART-SOUNDS IN THE REGION OF THE STERNUM *

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An examination of some thirty available works on physical diagnosis and diseases of the heart reveals that none of them call attention to the fact that there is a physiological difference in the quality of the heart-sounds underneath and in the neighborhood of the sternum, as compared with the sounds over the rest of the heart.

The quality of the sounds in this situation has undoubtedly been observed frequently, but the articles bearing on the subject all assume that the peculiarity is pathological. Laennec undoubtedly heard the sound and described it in his treatise on diseases of the heart. He mistook it at first for pericardial friction, but subsequently decided that this was not the case and considered it an association of cardiac hypertrophy.

F. J. Brown described the sound in 1856 as a "chisel" sound. He also considered it a pathologic manifestation and associated it with dyspepsia.

Angel Money noted it in puerperal women in 1882 and suggested its association with friction between the two layers of the pericardium which he thought might or might not be altered in structure.

Sansom agreed as to its occurrence, but rejected Money's explanation.

Colbeck described it, in 1901, as occurring especially in connection with downward displacement of the heart in patients with emphysema. He notes, however, that it may occur under normal conditions.

Hare, in a brief note published in 1901, associates it with general debility and a feeble, irritable heart, and states that it is of frequent occurrence in the early stages of pulmonary tuberculosis.

The most careful studies of the sound have been made by Myer Solis-Cohen,[1] who describes it as a xiphosternal crunching sound. He suggests that it does not indicate a grave pathological condition, and that its association with enlargement of the heart and myocardial degeneration is to him its most significant feature.

Osler, in his brief discussion of Hare's paper, is the only observer, so far as I have been able to discover, who notes that the sound is found in the majority of healthy subjects.

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* Submitted for publication, June 9, 1914.

* From the Continuous Medical Service of the New Haven Hospital.

[1] The important literature may be found in his article, "A Xiphosternal Crunching Sound," Tr. Lehigh Valley Med. Assn., 1903. Am. Jour. Med. Sc., July, 1903.

This peculiar quality of the heart-sounds underneath the sternum and in its neighborhood seems to be worthy of emphasis because the phenomenon is a normal rather than a pathological one, and because under certain conditions it may be mistaken for a pathological sound and give rise to errors in diagnosis. I have noted the almost constant presence of the sound in all sorts of cases for a number of years, and recently have studied in detail some forty cases.

The sound occurs at all ages after infancy. I am not prepared to say that it never occurs in infants, because I have not examined a sufficient number to be certain of this point, but in those which I have examined the sound was not perceptible. After the age of 2 years I have seldom failed to find it.

The quality of the sound is, to my mind, best described in the great majority of instances as a superficial scratching sound. In a few instances it has a clicking or tapping quality. The scratching quality is present with both systole and diastole. Usually it is of brief duration and of equal intensity with both sounds. Occasionally the systolic scratch is the more intense; rather more frequently the diastolic. The sound appears to be decidedly superficial, does not resemble an endocardial murmur, but does strongly suggest a friction rub. It is uninfluenced by respiration, and in many instances change of position has little or no effect on its intensity. In about 50 per cent. of the cases it is somewhat intensified by the leaning forward position or by the right or left lateral position. In the left lateral position the maximum area of the sound sometimes shifts 1 or 2 cm. to the left.

The sound is usually heard best at the lower end of the sternum opposite the fifth and sixth costal cartilages, a little to the left of the median line. I have endeavored to get an average of its distribution by marking it out on the chest in a series of patients and transferring the markings to tracing-paper. By making a composite tracing as accurately as that can be done, it is found that in the average adult the area over which the scratching quality is readily appreciable measures about 13 by 9 cm.; in children, about 7 by 8 cm. In both instances the area to the left of the median line is greater than that to the right, and shows a tendency to project downward toward the apex of the heart. In hearts which are hypertrophied or dilated from disease the area is usually distinctly enlarged, averaging about 15 by 12 cm. In a vertical direction there is a good deal of variation in the situation of the area of scratching. It is practically always prominent just above the ensiform cartilage, and occasionally can be heard below the ensiform. It only occasionally passes upward above the angle of Louis. I wish to remark, however, that there is quite a wide range of variation and that I have charted only the area of maximum intensity of the sound because it shades off almost imperceptibly, and a certain degree of scratching quality can often be heard well beyond this area.

I am somewhat at a loss for an explanation of the sound. The writers who have regarded it as pathological have ascribed it to various known pathological lesions of the pericardium or heart, such as milk spots on the pericardium, dryness of the surface of the pericardium and ecchymoses into the subserous tissue. Others have regarded the sound as being of cardiac origin and associated with sclerosis of the coronary arteries and hypertrophy of the heart. Inasmuch as the condition is a physiological one, pathological explanations do not suffice to account for it. The sound is so much like a pericardial rub that one is tempted to assume that in spite of the presence of lubricating fluid in the pericardial sac there is still enough friction to produce sounds audible through the chest-wall. This explanation seems to me improbable, however, as in a patient with a tuberculous pericarditis with a very large effusion, I was still able to make out the scratchy quality to the heart sounds over the lower end of the sternum. A possible explanation which occurs to me is that the sound may be due to changes in the tension of the loose cellular tissue in the sterno-pericardial ligament which binds the anterior surface of the pericardium to the posterior thoracic wall, and which may be affected by the movements of the heart. I have no way of proving or disproving this hypothesis, nor is it one that can be readily elucidated by dead-house findings. The location of the ligament as seen in cross-sections and at necropsy seems compatible with this explanation.

The practical importance of the scratching, substernal sound lies in the fact that it apparently becomes intensified under certain circumstances. At least this seems to me to explain certain otherwise inexplicable phenomena. Six or seven years ago, before I had given very careful attention to the sound, a patient comatose from alcohol entered my ward in the hospital, and on physical examination I very carefully described a distinct to-and-fro pericardial friction. The next morning it had entirely disappeared. Since then I have occasionally met with cases in which the scratching quality was intensified and in which I was in doubt as to whether I was dealing with a physiological phenomenon or a beginning pericarditis. These were usually patients with some acute infection associated with dilatation of the heart. On the other hand, I am sure that I have once or twice mistaken a real pericardial rub for the substernal scratching sound, and in one instance I have been able to substantiate this by necropsy. I do not believe that the sound will ever be a frequent source of error, but I am confident that it will be an occasional one. For this reason I think it important that it should be recognized as a physiological phenomenon.

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BOOK REVIEW

Modern Medicine. Its Theory and Practice in Original Contributions by American and Foreign Authors. Edited by Sir William Osler, Bart., M.D., F.R.S., and Thomas McCrae, M.D. Second edition, thoroughly revised, illustrated. Lea and Febiger, Philadelphia and New York. Volume I, 1913; volume II, 1914.

The first and second volumes of "Modern Medicine" give many evidences of reediting and alteration which are sufficient to justify this new edition ot the well-known work and serve to enhance its value to the general practitioner. The contributors to these two volumes are in most instances those who prepared the articles of the first edition, though there are some changes and additions in the minor subjects, and the names of a few new authors appear in addition to the contributors to the original edition.

The arrangements of the contents of the first two volumes is in many respects different from that of the original edition. Into these two books has been condensed practically all the matter contained in the first three volumes of the old edition, and the System starts with the infectious diseases. The first volume contains sections on bacterial diseases, diseases of doubtful or unknown etiology, non-bacterial fungus infections and the mycoses. The second volume opens with the section on diseases caused by protozoa and animal parasites; then follow diseases due to physical, chemical and organic agents and diseases of metabolism; finally, the section on diseases of the respiratory system completes the volume. Not only has the sequence of the various sections been altered, but certain chapters have been entirely eliminated.

The historical survey of medicine by Sir William Osler, which formed the introduction to the original System, has been omitted.

There has been a reduction in the length of the sections which deal with the pathology of various different diseases, but discussions on diagnosis and treatment have been frequently lengthened and enlarged, so that the System should appeal particularly to the practicing physician, for whom it is most valuable. In order to obtain more space for such changes the articles on "Life-Insurance" and the chapter on "Inheritance and Disease" have been omitted. But these omissions seem justifiable since any one who wishes to study such subjects may refer to more extensive books and monographs.

In the classification of diseases there have been some noticeable changes; acute poliomyelitis is included among the bacterial diseases; pellagra is placed with the affections caused by chemical and organic agents. The chapters on typhoid fever by McCrae, those on lobar pneumonia by G. W. Norris and on tuberculosis by Baldwin, MacCallum and Brown, fill nearly half of one volume and have been so altered and revised that they are perhaps even more useful than the original articles in the first edition. The sections on the contagious diseases of childhood are well illustrated with useful plates. The chapters by Sir William Osler on syphilis give a most interesting and excellent summary of this subject so important and so difficult of study. In the treatment of this disease he emphasizes the value of the use of mercury. The diseases of metabolism are again admirably presented by Philip R. Hawk, Thomas B. Futcher, James M. Anders, George F. Still and Robert Hutchinson.

There are certain changes in the makeup of these books which have allowed of condensation of considerable reading matter in a smaller space, so that eventually the System will contain fewer volumes and be much less bulky. To do this the paper used is thinner than that originally employed, and though it is probably not so good, it makes a much lighter volume. The print, too, is large and clear, which makes the book more agreeable to read than the earlier edition.

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THE PULSE-FLOW IN THE BRACHIAL ARTERY

IV. REFLECTIONS OF THE PRIMARY WAVE IN DICROTIC AND MONOCROTIC PULSE-FORMS *

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It is well known that in most tracings from the peripheral arteries various secondary waves follow the principal or primary wave on the descending limb of the pulse. The chief of these is usually spoken of as the dicrotic wave; and when this wave can be distinctly felt by the palpating finger on the radial artery, the pulse is described as dicrotic. The typical dicrotic pulse is relatively common in typhoid fever, but it occurs not infrequently in all types of infection. It is also occasionally encountered in other conditions, as, for example in cardiac patients with badly broken compensation. Marked instances of dicrotism are frequently but not invariably associated with low blood-pressure. On the other hand, equally low blood-pressures occur without marked dicrotism. In this as in many other conditions the relation between blood-pressure, whether systolic or diastolic, and pulse form is but a loose one. Pulse form does not depend directly on blood-pressure, though the two are controlled by many factors in common.

During the past year I have studied the brachial flow in seven patients who presented definitely palpable dicrotic pulses. In all of these cases the records obtained showed characteristic variations from the usual pulse-flow in the brachial artery (Figs. 1 and 2). Instead of the step-like entrance of blood most commonly seen in normal tracings, the primary wave was immediately followed by a marked retrograde movement of blood in the artery, and this in turn was followed by a forward movement of blood due to the dicrotic wave. The smaller secondary waves so frequently seen on the normal pulse were absent. The tracings showed many points in common with those obtained from normal individuals who have taken nitroglycerin. The marked backflow in the brachial artery which occurs just after the entrance of the primary wave is present in both, and is, I believe, due

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to a reflection of the primary pulse-wave in the vessels of the arm. Certain differences between the nitroglycerin pulse and the dicrotic pulse of fever will be discussed later.

One of the characteristic features of sphygmographic radial tracings of the dicrotic pulse is the marked fall in the pressure-curve between the primary and the dicrotic waves, which may be so great that the tracing descends nearly as low as and occasionally even lower than it does at the end of the pulse-cycle. Our records show that this fall of pressure is associated with a backflow in the brachial artery. Hirschfelder[1] has based his classification of pulse-form on the degree of this fall after the primary pulse-wave, and Marey apparently assumes a similar classification. According to this view, all pulses which show a marked fall in the late systolic portion of sphygmographic tracings are grouped together, irrespective of the size of the dicrotic waves. In the present paper I shall designate as monocrotic a pulse which shows this marked fall of pressure in late systole without the presence of a prominent dicrotic wave. That this sudden fall of pressure is of greater importance than is the size of the dicrotic elevation is shown by the fact that variations in the latter often occur while the former remains constant. Thus von Kries[2] found that during

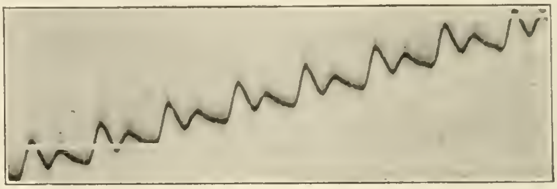

Fig. 1.—Common type of pulse flow in a palpable dicrotic pulse. The horizontal lines represent a difference in volume of 2 c.c. Vertical interruptions, ¼ second. Rate of flow, 2.1 c.c. per hundred c.c. arm substance per minute. Height of primary wave above the average rate of flow, 0.5 c.c.

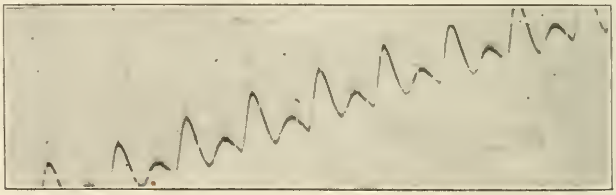

Fig. 2.—Unusually large primary wave (0.8 c.c.) with unusually marked backflow after this wave in a patient with dicrotic pulse. Rate of blood flow, 2.2 c.c.

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[1] Hirschfelder, A. D., Diseases of the Heart and Aorta, 1910, p. 44.

[2] Von Kries: Studien zur Pulslehere, Freiburg, 1892.

the height of amyl nitrite action, the radial pulse was monocrotic and that the dicrotic wave appeared and became more marked as the action of the drug wore off. Febrile patients may also show great variations in the size of the dicrotic wave while preserving the marked systolic fall, as may be seen in tracings published in Marey's "Circulation du sang" (Fig. 274). Further evidence of the intimate relationship which exists between the monocrotic pulse as above defined and the dicrotic pulse is also obtained by records of the brachial pulse flow. A typical monocrotic pulse is shown in Figure 3, and it will be seen that like the dicrotic pulse there is a very marked backward movement of the blood in the brachial artery just after the entrance of the primary pulse-wave. This record was obtained from a typhoid fever patient who, on other occasions, showed a typical dicrotic pulse. Nitroglycerin will produce in some individuals a monocrotic pulse, in others a dicrotic pulse. In still others the typical monocrotic flow pulse present at the height of the action later becomes a dicrotic pulse as the effect of the drug wears off, an observation analogous to that of von Kries on the effect of amyl nitrite on radial tracings. It is, I believe, quite certain that the typical dicrotic pulse and the typical monocrotic pulse (as above defined) are but subvarieties of a more general type in which the primary pulse-wave has a pointed form on account of the marked fall in the pressure tracing just after its entrance. This fall is accompanied by the exit of a considerable amount of blood from the larger arteries of the arm owing to a local reflection of the primary pulse-wave.

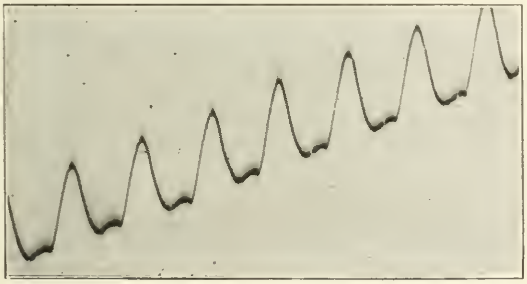

Fig. 3.—Marked monocrotic pulse.

In a previous paper[3] it was shown that nitroglycerin produces a marked backward movement of the blood in the brachial artery just after the entrance of the primary pulse-wave, and my collaborators and I have endeavored to explain this backward movement solely from the point of view of the changed conditions in the local vascular apparatus of the arm. It was assumed that a relaxed condition of the larger vessels of the arm allowed an unusually large primary wave to enter this region, and that the reflection of this wave by the relatively constricted finer arterioles was a sufficient explanation of the backward movement of blood in the brachial artery just after the entrance of the primary wave. An attempt to apply this explanation to the dicrotic pulse of fever encounters certain difficulties. In the accompanying table I have given the records from our seven febrile patients who presented definitely palpable dicrotic pulses. In addition to the brachial flow records, which give the average rate of blood-flow in the arm and the height of the primary wave above this average flow, I have determined the velocity with which the pulse wave moved down the arm, by making simultaneous pressure tracings of the radial and of the carotid or brachial pulse-curves. The blood-pressure records in the table were in most but not in all instances taken at the same time as the other records.

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[3] Hewlett, A. W.: Van Zwaluwenburg, J. G., and Agnew, J. H.: The Pulse Flow in the Brachial Artery, The Archives Int. Med., 1913, xii, 13.

PALPABLE DICROTIC PULSE

Patient	Diagnosis	Rate of Blood Flow per 100 c.c. Arm Substance per Minute, c.c.	Height of Primary Wave Above Average Inflow, c.c.	Velocity of Pulse Wave, cm.	Blood-Pressure	
					Max.	Min.
Mor.	Typhoid.....	2.5	0.5	840	120	76
Bus.	Typhoid.....	2.5-4	0.35-0.5	650	112	
V. H.	Typhoid.....	2.2	0.8	650	115	90
Barn.	Tuberculosis	2.5	0.45	750	130	
Mered.	Tuberculosis	2.0	.....	...	110	72
O'L.	Tuberculosis	2.5	0.6	830	95	
Bo.	Pneumonia..	1.7	0.4	870	108	57

It will be seen from this table that in all of the cases studied the average rates of blood-flow in the arm during marked dicrotism approached the lower level of the normal, which, by the method used, usually lies between 2 and 8 c.c. of blood per hundred c.c. of arm substance per minute. It is evident, therefore, that in cases of marked dicrotism there is no increased blood-flow in the arm, and consequently no marked dilatation of the finer arterioles in this vascular area. It cannot, of course, be inferred that there is likewise no vasodilatation of the arterioles in other important vascular areas and especially in the splanchnic region, for we know that an antagonism frequently exists between these vessels and those of the extremities.

It is likewise apparent from the table that the size of the primary pulse wave in the exquisite type of dicrotic pulse here studied is if anything less than the average. Thus in a series of twenty-five individuals showing various clinical conditions, exclusive of aortic insufficiency, the size of the primary pulse-wave as measured above the average rate of inflow varied from 0.4 to 1.1 c.c. with an average of

0.63 c.c.; while in our dicrotic pulses the primary wave varied from 0.35 to 0.8 c.c., with an average of 0.51 c.c. In this respect, therefore, the brachial flow in the dicrotic pulse differs from that present after the administration of nitroglycerin, for in the latter the size of the primary wave is almost invariably considerably larger than what was normal for the individual studied. It is thus evident that in the dicrotic pulse of fever with its close resemblance to the nitroglycerin pulse we lack one of the features which led us to attribute the latter chiefly to changes in the local blood-vessels of the arm itself. In the dicrotic pulse the primary wave which enters the arm is not unusually large.

The condition of the arteries of the arm is not easily determined, but from palpation one gets the impression that in the dicrotic pulse of fever the size of these vessels does not differ greatly from the normal. Furthermore, the rate of propagation of the pulse-wave is not markedly or constantly changed from the normal, which varies from about 670 to 1,000 cm. per second, with an average of about 825 cm.[4] Since the rate of propagation depends largely on the elastic coefficient of the arterial walls, it would appear that a marked reduction in the elastic coefficient of the larger vessels of the arm is not a constant and essential factor in the production of the dicrotic pulse of fever.

Our records indicate, therefore, that marked instances of the febrile dicrotic pulse are associated with moderate to marked constriction of the finer arterioles in the arm and with the entrance into the arm of primary waves of normal or subnormal size. So far as we can judge, there are no marked and constant changes in the condition of the larger arteries of the arm. It seems difficult to explain the marked oscillations in the brachial column of blood from these data alone and we are inclined to refer the dicrotic pulse of fever, in part at least, to cardiovascular changes outside of the arm. Before discussing the character of these changes, however, we wish to bring further evidence in favor of the view that a reflection of the primary pulse-wave in the arm is an important factor in the production of the pointed primary wave, which is such a prominent feature of dicrotic and monocrotic pulse-forms.

RELATION OF POINTED PULSE-FORMS TO REFLECTIONS OF THE PRIMARY PULSE-WAVE IN THE ARM

The view that dicrotism is associated with marked reflection of the primary pulse-wave in the arm stands in apparent contradiction to the commonly accepted view that dicrotism is most frequently encountered in conditions of vascular relaxation. According to this view, as described for example by Hirschfelder, the flow of blood through the arterioles is so rapid that a very marked fall of arterial pressure occurs even during the systolic portion of the pulse. Our records show that so far as the arm is concerned no such rapid flow of blood is present.

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[4] Friberger, R.: Ueber die Pulswellengeschwindigkeit bei Arterien mit fühlbarer Wandverdickung, Deutsch. Arch. f. klin. Med., 1912, cvii, 280.

Indeed, if the arterioles of the arm are markedly dilated and the local blood-flow is very fast, the pulse tends to lose its dicrotic character. Very rapid local flows in the arm can be produced by having an individual remain in a tub of very hot water until free perspiration is established and the body temperature is slightly elevated. The volume pulse then assumes the form shown in the lower curves of Figures 4 and 5. It is characterized by a gradual fall in the tracing and by a dicrotic wave which occurs relatively high on the tracing, both being characteristics opposite to those which are present in typical dicrotism.

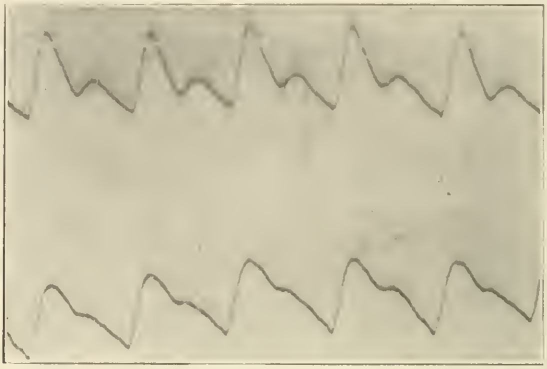

Fig. 4.—Volume pulse of the arm. Effect of constricting the arteries at the wrist after taking a very hot bath. Lower tracing without constriction, upper with constriction.

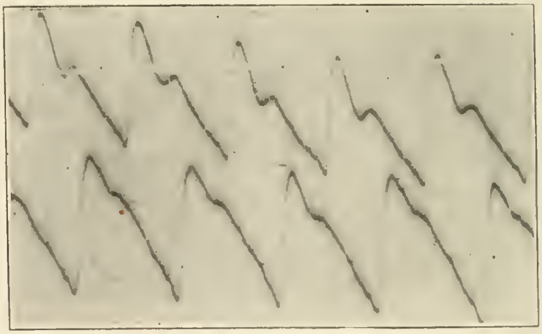

Fig. 5.—Volume pulse of the arm. Effect of constricting the arteries at the wrist after taking a very hot bath. Lower line without constriction, upper with constriction.

Veiel[5] observed similar changes in the radial pressure pulse after a hot bath.

According to Stewart,[6] a compression of the radial pulse below the sphygmograph will cause the typical pulse of aortic insufficiency to lose its collapsing character. According to Marey (Fig. 138), on the other hand, such a compression may increase the dicrotism of a normal pulse. It is well known that sphygmographic tracings are liable to be influenced by gross technical errors, and we have therefore sought to make similar observations on the volume pulse of the arm when the arteries about the wrist were compressed. This was accomplished by placing a pressure cuff about the wrist within the plethysmograph and applying pressures of 200 mm. or more of mercury. The rubber tube to the pressure cuff was carried out of the plethysmograph, and it was therefore possible to obtain alternate tracings of the volume pulse or of the brachial flow when the wrist arteries were compressed and when they were left open.

The effect of obstructing the arteries at the wrist varied in different individuals and in the same individual at different times. In many cases the compression of the wrist produced little effect on the form of the volume curve; but in no case have we encountered any marked tendency toward diminished dicrotism as a result of such pressure. On the other hand, a number of records were obtained in which an increased dicrotism appeared when the wrist arteries were compressed. This effect was obtained with considerable regularity when the very rapid blood-flow after a hot bath was obstructed at the wrist by a pressure cuff (Figs. 4 and 5). One of our typhoid fever patients who had shown a typical dicrotic pulse on several occasions showed on one examination a rapid blood-flow through the arm (10 c.c. per hundred c.c. of arm substance per minute) and a pulse that was not definitely dicrotic. Compression of the wrist arteries slowed the blood-flow to 3 c.c., and at the same time the pulse took on a dicrotic character (Fig. 6).

Since the pressure curves from arteries in the arm follow nearly the same course as do the volume curves, it seems probable that similar changes in the pressure curves were present. These observations furnish further evidence in support of the view that one of the important factors favoring the production of a sharp fall in the volume (and pressure) tracings immediately after the primary wave

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5. Veiel, E.: Ueber die Bedeutung der Pulsform, Deutsch. Arch. f. klin. Med., 1912, v, 249.

6. Stewart, H. A.: Experimental and Clinical Investigation of the Pulse and Pressure Changes in Aortic Insufficiency, THE ARCHIVES INT. MED., 1908, i, 102.

is a constriction of the finer arterioles of the arm which tends to cause a reflection of the primary wave in this region.

THEORIES OF THE POINTED CHARACTER OF THE PRIMARY WAVE IN DICROTIC AND MONOCROTIC PULSE FORMS

We have seen that the common and probably the most important feature of the dicrotic and monocrotic pulse-forms is the marked backward movement of blood in the brachial artery just after the primary pulse-wave, which movement gives rise to a pointed form of this wave in both volume and pressure records. Although present data hardly permit a satisfactory explanation of this pointed type of pulse, it may be of interest to review certain theories in the light of our observations and to suggest possible explanations for this type of pulse. I have shown previously that in the nitroglycerin pulse the marked backward movement of blood in the brachial artery during late systole could not be attributed solely to a settling back of the blood-column against the aortic valves. In that type of pulse as well as in those discussed in the present paper, the movement backward in the brachial artery is altogether too great to be accounted for in this manner. The only explanation which seems possible for this backward movement is that it is due to a reflection of the primary wave in the arm. New evidence in favor of this view is given in the present paper, and it is supported by a comparison of volume curves from the arm and from the hand, which are published elsewhere.[7]

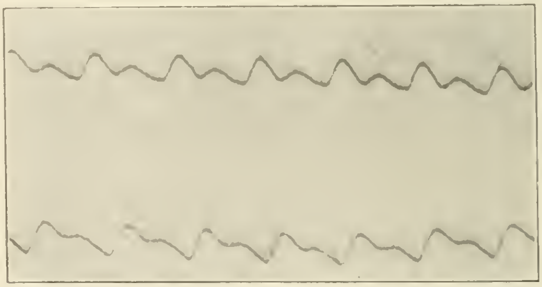

Fig. 6.—Volume pulse of the arm. Effect of constricting the arteries at the wrist in a patient with typhoid fever. The lower curve without constriction. Blood flow, 10 c.c. The upper tracing with constriction. Flow reduced to about 3 c.c.

In the nitroglycerin pulse an unusually large primary wave enters the arm, and in a previous paper I sought to explain that type of pulse solely on the basis of changes in the arm vessels. In the dicrotic pulse of fever, however, such an explanation encounters the difficulty that the primary wave may be unusually small and yet the backflow in the brachial artery may be relatively large. For this and other reasons it does not seem possible to account for the peculiar features of the dicrotic pulse solely on the basis of circulatory changes within the arm. It seems to us necessary to assume that changes in the cardiac or vascular mechanism outside of the arm play an important part both in the production of the backward movement of late systole and in the size and time of onset of the dicrotic wave.

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[7] Hewlett, A. W.: Reflexionen der primären Pulswelle im menschlichen Arme, Deutsch. Arch. f. klin. Med., 1914, cxvi, 237.

Just what these conditions are is difficult to determine. Since the primary wave entering the arm interferes to a greater or lesser extent with its own reflection, a separation of these two in point of time would tend to make the reflected wave more evident on our tracings. Such a separation might be produced by a relatively slow rate of propagation of the pulse wave in the arm; but this, as we have seen, does not appear to be a constant finding in the types of pulse here studied. A short primary wave due to a rapid systolic emptying of the heart would also diminish this interference, and in cases of tachycardia as well as certain extrasystoles the frequently observed pointed character of the pulse may be due to this cause. Whether or not this factor plays a part in the backflows here described we do not know.

The known facts in regard to these pulse-forms may also be explained in accordance with the views of von Kries. This author concluded from theoretical studies on wave motion in elastic tubes and from comparisons of the pressure pulse and the tachogram of the arm of man that reflections of the primary pulse waves usually take place in the arm. My tracings show that under certain conditions such reflections produce well-marked backflows just after the entrance of the primary pulse wave. It is highly probable that similar reflections occur at numerous other points in the vascular tree and that these complicated systems of reflected waves return to the aorta and may then be propagated as centrifugal waves into various arteries. Under normal conditions these numerous and complicated systems of reflected waves tend to neutralize one another by interference, and the pulse approaches the type produced by the intermittent expulsion of fluid into an elastic bag with a narrow outlet. Von Kries explained the changes in pulse form produced by amyl nitrite by assuming that, owing to vascular dilatations in parts of the body other than the arm, certain of these reflected waves were absent and that the absence of these reflected waves which would otherwise have entered the arm between the primary and the dicrotic waves was the cause of the marked fall in pressure after the primary wave.

A similar explanation may be given for the pointed character of the primary wave in the dicrotic and monocrotic pulse-forms of fever, and it may well play an important part in the production of other pointed pulse-forms. A vasodilatation of certain vascular areas outside of the arm may diminish or eliminate certain reflected waves which would otherwise enter the arm between the primary and the dicrotic waves. This would allow the reflection of the primary wave in the arm to become apparent on our tracings as a negative blood-flow in the bracial artery. Knowing the rate of propagation of waves in the arterial system it is possible to estimate the distance which must be traveled in order that waves reflected from other parts of the vascular apparatus should enter the arm shortly after the primary wave. Such a calculation shows that these waves would be reflected from points lying in the neighborhood of 35 to 60 cm. beyond the points of origin of the subclavian arteries. The most important vascular areas lying at about this distance are the abdominal vessels. It is possible, therefore, that the pointed form of the primary wave in the dicrotic and monocrotic pulse of fever may be due in part at least to an unusual dilatation of the splanchnic vessels. This hypothesis is in accord with many statements concerning the conditions which produce these pulse-forms. Thus Cushny[8] states that amyl nitrite produces a more marked dilatation of the vessels in the head and the abdomen than in those of the extremities. Fever is likewise generally supposed to cause a dilatation of the splanchnic vessels. The frequent but not invariable association between low blood-pressure and dicrotism may be due to the fact that while dilatation of the splanchnic vessels frequently lowers the blood-pressure it may fail to do so owing to the constriction of vessels in other parts of the body. A constriction of the arterioles in the arm would tend to maintain the blood-pressure and at the same time it would increase the local reflection, provided the larger arteries of the arm were not correspondingly constricted.[3]

CONCLUSIONS

1. In febrile patients showing palpable types of the dicrotic pulse, the brachial flow curves show characteristic changes. These consist in a marked backflow in the brachial artery just after the entrance of the primary pulse-wave and in an inflow and outflow with the dicrotic wave.

2. In the form of pulse here described as monocrotic there is also a marked outflow just after the primary pulse-wave, but no marked dicrotic wave follows. This type of pulse is closely related to the

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[8] Cushny, A. R.: Pharmacology and Therapeutics, 1906, p. 465.

typical dicrotic pulse. The primary wave in each has a pointed character.

3. Other things being equal, the pointed character of the pulse in the arm is increased by vasoconstriction of the arm arterioles and is diminished by their relaxation.

4. We have been unable to explain all forms of the pointed pulse satisfactorily solely on the basis of local vascular changes in the arm; that is, constriction of the arterioles and relaxation of the larger arteries. In many, if not in all cases, factors outside of the arm play a part in their production.

5. Among such possible factors are (1) a brief systolic output from the heart and (2) a dilatation of certain vascular areas outside of the arm, the most important being the splanchnic vessels.

THE TOTAL NON-PROTEIN NITROGEN AND THE UREA OF THE BLOOD IN HEALTH AND IN DISEASE, AS ESTIMATED BY FOLIN'S METHODS *

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Ever since the time Prévost and Dumas,[1] who in 1823, first demonstrated an increase of the urea of the blood after extirpation of the kidneys in animals, the total non-protein nitrogen and the urea of the blood have been the subject of repeated investigations, and have been accorded considerable importance in the diagnosis and prognosis of Bright's disease. Owing, however, to the fact that the methods employed have been various and more or less subject to error, the results obtained have been conflicting. This may be readily seen when we find that the total non-protein nitrogen in the normal person is given as anywhere from 25 to 60 mg. per hundred c.c. of blood. The brilliant methods recently devised by Folin[2] render possible the accurate estimation of these substances in a small amount of blood, from 2 to 5 c.c. sufficing for all the analyses. The increase in accuracy depends on an improved method of removing the proteins from the blood, and the use of Nessler's solution makes it possible to work with small quantities of blood.

The term "non-protein nitrogen" explains itself. It includes all the nitrogenous substances remaining after the removal of the proteins by precipitation, in the case of Folin's method by means of methyl alcohol and later by zinc chlorid. Other names for it are "incoagulable nitrogen," "filtrate nitrogen," "rest," "waste" or "retention nitrogen."

Folin's method for total non-protein nitrogen is essentially a "micro-Kjeldahl" process, in which the ammonia after neutralization of the products of digestion is blown over into a collecting vessel by a current of air instead of by distillation, and is estimated by the use of Nessler's solution and the colorimeter as in water analysis. His method for urea depends on the quantitative breaking down of this substance to ammonia at a temperature of 150 C., and its subsequent estimation in the same manner as the total nitrogen. The figures

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* From the Department of Medicine, Yale University Medical School.

* Read in abstract at the meeting of the Association of American Physicians, May 13, 1914.

1. Prévost and Dumas: Ann. de chimie et de physique, 1823, xxiii, 90.
2. Folin, O., and Denis, W.: Jour. Biol. Chem., 1912, xi, 527.

obtained represent urea estimated as nitrogen, and include the ammonia nitrogen, which, however, is so small in normal blood, and presumably in most forms of disease, as to be negligible.

These new methods of Folin open up great possibilities both in the line of experimental research and in clinical work, and promise to throw new light on many problems connected with the function of the kidneys.

The present investigation was undertaken with the view of determining the non-protein nitrogen and the urea of the blood in a large series of miscellaneous diseases, paying special attention of course to affections of the kidneys. In this way it was hoped to gain a more exact idea as to the diagnostic and prognostic value of such determinations. The figures of previous investigators along these lines, of whom Strauss,[3] Hohlweg[4] and Widal[5] are especially deserving of mention, are not comparable to those obtained by Folin's methods, for reasons already given. The work of the French school is especially inaccurate, depending on the very faulty hypobromite method for the urea estimation, a method which Rowntree and Fitz[6] have shown to involve an error of from 10 to 60 per cent. We shall consider the results obtained by others working with the new Folin methods later on.

Before passing to a detailed account of our work, however, we cannot refrain from alluding to a practice of which H. Strauss has been guilty. This author, to whom we owe so much in the investigation of Bright's disease, has published as his own[7] a slight modification of Folin's method for total nitrogen, giving Folin credit only for devising the method of removing the albumin. His method is word for word the same as Folin's up to the stage of combustion, where he substitutes an ordinary Kjeldahl determination on a large amount of filtrate for Folin's "micro-Kjeldahl" process. His offense is rendered more flagrant by the fact that he went to Folin's laboratory to learn Folin's method before publishing his article.

TECHNIC

We have followed very closely the original technic as described by Folin and Denis,[2] and learned by one of us (Tileston) in Professor Folin's laboratory. The material has consisted of whole blood, oxalated, and the results have been calculated as milligrams of nitrogen per 100 c.c. of blood. In order to avoid the effects of the ingestion of protein, the blood has been drawn in the forenoon when possible. In cases with very large amounts of nitrogen we have found it advantageous to take 2 c.c. instead of 5 c.c. of the filtrate for analysis, and to dilute the specimens with water before adding Nessler's solution, thus avoiding turbidity. The determinations have been made in duplicate, with further controls in case the figures did not agree closely. The methods have proved accurate and reliable in our hands, and for any one with an eye for color, are not much more difficult to learn than the ordinary Kjeldahl method.

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[3] Strauss, H.: Die chronische Nierenentzündungen in ihrer Einwirkung auf die Blutflüssigkeit, Berlin, 1902; also Deutsch. Arch. f. klin. Med., 1912, cvi, 219.

[4] Hohlweg, H.: Deutsch. Arch. f. klin. Med., 1911, civ, 216.

[5] Widal, F.: Bull. et mém. Soc. méd. d. hôp. de Paris, 1911, xxxii, 627. (And many other articles.)

[6] Rowntree, L. G., and Fitz, R.: The Archives Int. Med., 1913, xi, 258.

[7] Strauss, H.: Ztschr. f. Urol., 1913, vii, 287.

THE TOTAL NON-PROTEIN NITROGEN AND THE UREA IN HEALTH

Folin and Denis,[8] working with the new methods, found in a series of sixteen healthy adults the total non-protein nitrogen varying within narrow limits, from 22 to 26 mg. per 100 c.c. of blood, while the urea nitrogen was exactly half as much, from 11 to 13 mg. The blood was taken in the forenoon, from three to six hours after breakfast. The figures given by previous investigators, as already mentioned, are all too high by reason of faulty methods.

TABLE 1.—Normal *

Case	Age	Total Nitrogen (mg.)		Urea Nitrogen (mg.)	
		After fasting 12 hrs.	2½ hrs. after heavy meal	After fasting 12 hrs.	2½ hrs. after heavy meal
D. G. R.	24	22.9	25.9	12.0	13.6
L. V. G.	26	25.0	27.9	13.6	14.5
E. M. J.	27	23.7	29.5	12.6	15.1
C. W. C.	29	23.8	32.3	14.1	20.9
W. T.	39	23.2	26.4	12.3	13.1

* In this and the following tables, the figures for total nitrogen and for urea nitrogen represent milligrams per 100 c.c. of blood.

In order to study the influence of diet, we examined five healthy male adults, early in the morning after a twelve-hours fast, and again in the afternoon of the same day, two and one-half hours after a meal containing a large amount of meat. The urine was normal in all these persons. As will be seen by a glance at Table 1, our fasting figures agree very closely with those of Folin, the nitrogen varying from 23 to 25 mg., and the urea from 12 to 14 mg. The effect of a heavy protein meal is seen to be a rise in the non-protein nitrogen of from 2.9 to 8.5 mg., averaging 4.7 mg., and a rise in the urea averaging

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[8] Folin and Denis: Jour. Biol. Chemistry, 1913, xiv, 33.

2.5 mg. One of our subjects (C. W. C.), who ate a great deal of meat, shows such a divergence from the others that an abnormality of the renal function seems possible, though the urine was negative. Not only did the nitrogen rise to a high figure after the meal (32 mg.), but the urea amounted to considerably more than one-half the total nitrogen (65 per cent.).

The results show the need of considering the ingestion of food in interpreting the results of blood analysis, and the advisability of taking the blood either fasting or before the chief meal of the day. Further studies along these lines are desirable.

THE TOTAL NON-PROTEIN NITROGEN AND THE UREA IN DISEASE

One hundred and forty-two cases of disease of one sort or another have been studied. For the purpose of classification they have been divided into eleven groups, as follows: (1) chronic nephritis; (2) other diseases of the kidneys and of the genito-urinary tract; (3) lead-poisoning; (4) the complications of pregnancy; (5) acute intestinal obstruction; (6) diseases of the heart and aorta; (7) the acute infections; (8) syphilis; (9) tuberculosis; (10) diseases of the nervous system; (11) miscellaneous diseases. These will be taken up in order.

For practical purposes figures for nitrogen below 30 mg. may be considered normal; those from 30 to 35 slightly, from 35 to 50 considerably, and from 50 to 100 greatly increased. One hundred milligrams or more, as we shall see later, constitutes a very dangerous elevation of the waste nitrogen. In the case of urea nitrogen, anything over 16 mg. is probably abnormal, and above 25 mg. considerably increased.

In the tables the figures for total nitrogen and urea nitrogen represent milligrams per hundred c.c. of blood. The abbreviations are explained under Table 2.

I. CHRONIC NEPHRITIS

A. *Chronic Interstitial Nephritis.*—In this disease we find figures varying all the way from normal to the enormous amount of 324 mg. for the nitrogen and 237 mg. for the urea. The seventeen cases fall naturally into two groups, those with normal or slightly elevated figures, and those with a marked increase. In the former, with one exception (Case 7), we have found a complete absence of all symptoms referable to renal insufficiency, although the hypertension in some was of long standing. These patients were mostly of the elderly, arteriosclerotic type. In the group with high nitrogen and urea, more or less pronounced symptoms of uremia have been present in all, excepting the border-line case, No. 12, with 57 mg. nitrogen. Of the six patients with over 100 mg. of nitrogen, five died within a period of 35 days, the sixth (Case 13) who came into the hospital with well-marked symptoms of chronic uremia, and 80 mg. of nitrogen, became worse at first, and the retention nitrogen rose to 102 mg.; then improvement set in, and he left the hospital in fair condition at the end of two months, with a blood nitrogen of 70 mg. But the improvement was only temporary. He has just entered the hospital again with the old symptoms, and a blood nitrogen of 88 mg. fourteen weeks after the first examination.† Farr and Austin[9] report a still more striking case (No. 31 of their series) of uremia with delirum tremens in which the nitrogen fell from 181 mg. to 36 mg. under treatment, and the patient was discharged in good shape. The subsequent history of these patients would be interesting. Javal[10] states that such patients usually relapse and die within a few months, and reports[11] one patient surviving eight months and still alive, as extremely exceptional.

We have encountered no case of uremia of the asthenic type, that is, with weakness and apathy, and gastro-intestinal symptoms—which has not shown a very marked increase of retention nitrogen. The great importance of blood analysis in the diagnosis of uremia, and in the prognosis of chronic nephritis, is evident from these facts.

The single case of uremia without retention of nitrogen (Case 7) calls for a more detailed description. The patient, a woman 64 years old, entered the hospital in a stuporous condition, a few hours after an epileptiform attack. The urine was consistent with the diagnosis of chronic nephritis; the systolic blood-pressure was 205 mm. and fell to 185 after venesection. No more convulsions occurred and the next day the patient was better and clear mentally. Eleven days later she was discharged in good condition. There was a history of an attack of unconsciousness, probably without convulsions, ten months previously. In the absence of evidence pointing to epilepsy, alcoholism or syphilis, it seems reasonable to attribute the convulsion to nephritis. The non-protein nitrogen was 31.5, the urea 16.4 on the day of entrance; the phthalein output was 42 per cent. There was evidently no renal insufficiency. This case fits in with the epileptiform type of uremia described recently by Reiss,[12] in which there are convulsions exactly resembling those of epilepsy, with euphoria in the intervals, no increase in the non-protein nitrogen of the blood and no deficiency in renal function. Such cases have been described by other authors, and Strauss pertinently asks if they are to be considered as truly of uremic origin.

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† Note on reading proof: This patient died Aug. 11, 1914, of uremia, eighty-eight days after the nitrogen reached 100 mg.

[9] Farr, C. B., and Austin, J. H.: Jour. Exper. Med., 1913, xviii, 228.

[10] Javal, A.: Bull. et Mém. Soc. méd. d. hôp. de Paris, 1911, xxxii, 485.

[11] Mosny, Javal and Levy-Bruhl: Bull. et mém. Soc. méd. d. hôp. de Paris, 1911, xxxii, 374.

[12] Reiss, E.: Ztschr. f. klin. Med., 1914, lxxx, 97.

B. Chronic Diffuse Nephritis.—We have examined only two cases of this disease. The first (Case 18) showed anasarca at entrance, with large amounts of albumin in the urine. The total nitrogen was normal and the urea unusually low (35 per cent. of the total non-protein nitrogen); there were no uremic symptoms. The normal nitrogen figures frequently encountered in chronic diffuse nephritis have been ascribed to the hydremia or dilution of the blood. That this is not necessarily so was shown by later examination in this case after the edema had disappeared; the nitrogen was even lower than before. The second case (No. 19) entered the hospital with moderate edema, a normal blood-pressure and mild uremic symptoms. During his long stay the nitrogen gradually rose from 55 mg. to 67 mg. five weeks later, then rapidly to 174 mg. the day before his death from uremia, five months after the first examination. Meanwhile the blood-pressure rose to over 200 mm. and he developed a high grade of albuminuric retinitis. At the autopsy there was a marked chronic nephritis with hypertrophy of the heart (weight 600 gm.) and moderately advanced tuberculosis of the lungs. The combined weight of the kidneys was 300 gm. Microscopically, there was very extensive glomerular disease; the tubules were dilated and filled with casts, and here and there showed degeneration and desquamation of the epithelium; there was a dense infiltration of the interstitial tissue with lymphoid cells.

The single case of amyloidosis (No. 20) showed practically normal figures.

C. Acute Nephritis.—Of this disease we have but one example to report, a case of acute arsenical poisoning ending in recovery (Case 21, Table 3). Two days after taking a large dose of Paris green the nitrogen was 70 mg. and the urea 58 mg. With convalescence the figures sank to normal. There was no edema. This case is comparable to the experimental uranium nephritis investigated by Frothingham,[14] who found a marked increase of retention products at the height of the disease.

Before leaving the subject of Bright's disease we wish to discuss the relationship of albuminuric retinitis to retention. Widal[15] claims that this condition is always an evidence of azotemia, which is his

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13. Strauss, H.: Deutsch. Arch. f. klin. Med., 1912, cvi, 219.

14. Frothingham, C., Fitz, R., Folin, Otto, and Denis, W.: The Relation Between Non-Protein Nitrogen Retention and Phenolsulphonephthalein Excretion in Experimental Uranium Nephritis, The Archives Int. Med., 1913, xii, 245.

15. Widal, F.: Presse méd., 1912, xx, 973.

TABLE 2.—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
1	M	41	Chronic interstitial	180	66	1,300	1.017	S. P. T.
2	F	62	Chronic interstitial	195	. .	600	1.023	S. P. T.
3	F	52	Chronic interstitial	210	40	(?) 390	1.019	S. P. T.
4	F	75	Chronic interstitial	210	38	460	1.019	S. P. T.
5	F	66	Chronic interstitial	220	38	S. S.	1.012	S. P. T.
6	M	65	Chronic interstitial	200	. .	S. S.	1.030	S. P. T.
7	F	64	Chronic interstitial	205	42	1,050	1.020	V. S. T.
8	F	46	Chronic interstitial	180	50	520	1.019	V. S. T.
9	F	61	Chronic interstitial	250	. .	S. S.	1.010	S. P. T.
10	F	63	Chronic interstitial, with albuminuric retinitis	185	. .	2,040	1.018	V. S. T.
11	F	53	Chronic interstitial	240	. .	2,000	1.007	S. P. T.
12	F	24	Chronic interstitial, with cholelithiasis	. . .	80	650	1.014	S. T.
13	M	48	Chronic interstitial, with uremia	240	22 . . 26	530	1.016	V. S. T.
14	M	25	Chronic interstitial with albuminuric retinitis	212	0.0	279+	1.011	0.8%
15	M	55	Chronic interstitial, with uremia	210	0.5	730	1.010	S. T.
16	M	22	Chronic interstitial, with uremia	180	0.5	1,030	1.014	S. T.
17	M	25	Chronic interstitial with uremia	175	0.0	S. S.	1.010	T.
18	F	20	Chronic diffuse	132	43	420	1.030	2.0%
19	M	25	Chronic diffuse	146	5	960	1.016	0.4%
				210	0.0	1,440	1.010	H. T.
20	M	44	Amyloidosis	. . .	55	S. S.	1.026	S. T.

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* The following abbreviations are employed in this and the following tables: S. S.
S. T. = slight trace, T. = trace, H. T. = heavy trace, W. B. C. = white blood-cor-
Esbach method, and represent percentages.

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Few hyaline casts; W. B. C.	Mixed	23.0	11.4	Early process in kidneys. Wassermann +. No other indications of syphilis.
Numerous hyaline casts: few R. B. C.	Mixed	23.2	11.1	Recent attack of cardiac decompensation. No sign of renal insufficiency.
Rare hyaline cast; few W. B. C.	Mixed	27.4	13.4	
Few hyaline, granular casts; W.B.C.	Mixed	28.2	14.6	No symptoms.
Pus	Moderate protein Poor protein, 1 day	29.0 25.3	16.1	Complicated with cystitis. Acute pyelitis several months ago. Cerebral hemorrhages 1 year and again 1 month ago.
....	Mixed	29.7	16.1	Entered for epistaxis. No sign of renal insufficiency.
Rare hyaline casts; W. B. C.	Mixed	31.5	16.4	One epileptiform convulsion a few hours previous. Prompt recovery after venesection, without any more fits.
Occasional coarse granular cast; many W. B. C.	Soft	31.7	15.4	Subacute appendicitis recently. Early process in kidney.
Very few hyaline casts; some R.B.C.	Mixed	31.7	16.7	Blood-pressure over 200 for past 4 years. Never any symptoms of uremia.
Occasional hyaline cast; many R.B.C.	Mixed	32.0	17.7	Albuminuric retinitis of moderate intensity, one year's duration.
Rare hyaline cast	Mixed	35.3	....	Hypertension for several years. No sign of renal insufficiency.
Hyaline, occasional granular casts; W. B. C.	Soft Low protein, 6 days	57.5 24.2	27.7 12.5	Recent attack of gall-stone colic. No symptoms of uremia.
Hyaline, granular casts; R. B. C.; W. B. C.	Mixed	79.5 102.2 70.5 87.5	55.1 79.5 40.4 53.1	4/29/14 5/15/14 6/21/14 8/ 8/14 Lead poisoning probable. Symptoms of chronic uremia. Left hospital improved after 2 mos. Returned in 6 weeks in same condition as before.
Hyaline, granular casts; R. B. C.; W. B. C.	Milk	151.2	109.4	Probably secondary contracted kidney. Slight edema, headache, vomiting, blurred vision. Died 2 days later.
Hyaline, granular casts; many R. B. C.; W. B. C.	Mixed	188.7 286.2	141.0 191.0	4/23/14 5/13/14 Symptoms of chronic uremia at entrance. Died uremic 35 days after first analysis.
Few hyaline, granular casts; R.B.C. W. B. C.	Low protein, 5 da., mixed, 3 days	220.0 232.5 324.0	143.0 184.0 237.0	3/10/14 3/13/14 3/27/14 Entered with symptoms of chronic uremia. Second entrance 3/27/14. Died in coma 2 days after last analysis. Necropsy: small granular kidneys, combined weight, 105 gm.
Few hyaline, granular, epithelial casts; W. B. C.	Liquid	313.0	205.0	Died next day in coma, preceded by convulsions.
Many hyaline, granular casts; R.B.C. W. B. C.	Soft Salt free, 5 weeks	26.7 22.6	10.7 8.0	2/13/14 3/19/14 Considerable edema at first analysis; none at second. Discharged improved, 4/29/14.
Many hyaline, granular casts; R.B.C. W. B. C.	Mixed	55.2 67.0 140.6 173.7	31.5 49.0 113.1 131.3	1/26/14 3/ 7/14 5/16/14 5/20/14 At first admission, occasional slight symptoms of uremia, headache, dizziness, nausea, vomiting. Moderate general edema. Tuberculosis of lungs, 2d stage. Second admission 5/9/14, with pronounced uremic symptoms and albuminuric retinitis. Died uremic, 5/21/14. Necropsy showed very extensive disease of glomeruli, tubules and interstitial tissue.
....	Mixed	33.9	15.3	Enlarged liver and spleen; osteomyelitis with discharging sinuses for 1 year.

= single specimen, S. P. T. = slightest possible trace, V. S. T., = very slight trace; puscles, R. B. C. = red blood-corpuscles; the figures for albumin were obtained by the

TABLE 3.—Other Diseases of—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
21	M	48	Acute nephritis	85	48	590	1.013	V. S. T.
22	M	58	Chronic passive congestion	128	70	450+	1.022	S. P. T.
23	M	54	Chronic passive congestion	170	49	850	1.024	V. S. T.
24	M	58	Chronic passive congestion	120	50	1,660	1.011	S. P. T.
25	M	45	Chronic passive congestion	90	58	620	1.024	V. S. T.
26	M	47	Chronic passive congestion	115	53	500	1.023	S. T.
27	M	21	Chronic passive congestion	110	75	1,680	1.017	S. P. T.
28	F	16	Chronic passive congestion	...	..	S. S.	1.040	H. T.
29	F	27	Pyelitis	118	80	1,900	1.004	S. P. T.
30	F	19	Pyelitis	112	30	1,340	1.015	V. S. T.
31	M	47	Hypernephroma	125	..	1,220	1.018	S. T.
32	M	62	Stricture of urethra, with acute ascending infect.	180	..	S. S.	1.030	H. T.
33	M	63	Stricture of urethra, with acute ascending infect.	...	..	S. S.	1.020	T.
34	M	69	Tuberculosis of genitourinary tract	88	10	1,100	1.014	S. T.
35	M	86	Hypertrophied prostate with retention	85	..	.....	.....	.....
36	M	77	Hypertrophied prostate with retention	142	60	S. S.	1.022	T.
37	M	64	Carcinoma, prostate and bladder	110	10	1,180	1.017	S. T.
38	M	70	Carcinoma, bladder	...	0.0	.....	.....	.....

—Kidneys and Genito-Urinary Tract

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Hyaline, granular, pus casts; R. B. C. W. B. C.	Soft	69.7 29.5	57.7 15.7	4/25/14 Large dose of Paris green taken 5/18/14 2 days previously. Recovered.
Occasional hyaline cast; W. B. C.	Karell	24.6	13.7	Mitral regurgitation; cardiac decompensation; marked edema.
Hyaline, granular casts; R. B. C.; W. B. C.	Karell	25.0	13.8	Aortic regurgitation; luetic aortitis.
Hyaline casts; W. B. C.	Soft	30.6	16.9	Mitral regurgitation and chronic cardiac decompensation.
Few hyaline, granular casts; W. B. C.	Mixed	31.7	15.1	Mitral stenosis; failure of compensation without edema.
Many hyaline casts; R. B. C.; W. B. C	Karell	34.4	17.5	Edema; extreme cyanosis; died 2 days later.
Hyaline casts; few R. B. C.; W. B. C.	Soft	42.2	25.4	Mitral stenosis and regurgitation with decompensation.
Many hyaline, granular casts; W.B.C.	Soft	67.7	43.9	Blood taken a few hours before death from failure of compensation, secondary to mitral disease.
Occasional hyaline cast; pus	Soft	26.5	14.9	Acute febrile attack.
Rare hyaline cast; R. B. C.; pus	Liquid, soft	70.7 40.2	51.0 29.2	6/16/14 Complicated with pyelonephrosis 6/26/14 at entrance.
Many R. B. C.; W. B. C.	Mixed	33.5	18.2	Tumor involving nearly all of left kidney. Removed later at operation.
Much pus	Liquid	91.2	61.2	Died next day. Necropsy: extravasation of urine; gangrenous cystitis with perforation of bladder; purulent pyelitis.
Many R. B. C. and W. B. C.	Soft	209.0	172.0	Hemorrhagic cystitis with putrid urine. Died same day of asthenia; no convulsions.
R. B. C.; very many W. B. C.	Soft, with meat once daily	31.4	19.1	Entered hospital for retention. Tubercle bacilli in urine. Location of disease uncertain. Died 8 days after.
....	Liquid	50.0	37.6	Prostatectomy next day. Died 5 days later.
R. B. C.; W. B. C.	Mixed Low protein	48.7 30.5	37.6 17.2	3/13/14 Prostatectomy 5 days after second 4/ 1/14 analysis Recovered.
R. B. C.; pus.......	Mixed	41.0	24.3	Suprapubic drain in bladder before blood examination.
....	Soft	58.2 99.5	45.5 69.7	4/10/14 Operation on second day after 4/15/14 second analysis. Bladder filled with growth. Died 4 days later.

appropriate name for a marked increase of the nitrogenous waste products. That this is not always so is shown by our Case 10, in which a moderate grade of albuminuric retinitis of a year's duration was accompanied by normal figures for nitrogen and urea. Where the eye condition was advanced, however, we too have found marked azotemia.

II. OTHER DISEASES OF THE KIDNEYS AND OF THE GENITO-URINARY TRACT

A. Chronic Passive Hyperemia of the Kidneys.—In this condition we have found either normal or moderately elevated figures, even in cases with extreme congestion (cf. Case 26). The one exception was a young girl with very marked failure of compensation; the blood was taken only a few hours before death, and showed 70 mg. of nitrogen. No autopsy was permitted. So far as can be judged from a small series of seven cases, the absence of retention is the rule in pure passive hyperemia, and its presence would indicate a complicating nephritis or possibly infarction.

TABLE 4.—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
39	M	27	Lead colic	108	..	650	1.025	T.
40	M	26	Lead colic	160	..	675	1.030	S. P. T.
142	M	31	Lead colic	137	51	950	1.021	S. P. T.
41	M	35	Lead palsy	153	50	1,360	1.018	V. S. T.
42	M	36	Lead colic	188	..	620	1.022	0.05%
43	M	34	Lead colic	...	..	S. S.	.....	S. P. T.
44	M	63	Chronic plumbism, with chronic interstitial nephritis and uremia	205	..	S. S.	1.020	0.2%

B. Pyelitis.—In one of the two cases examined there was a considerable retention, amounting to 71 mg. of nitrogen. This case was complicated with pyonephrosis; the retention almost disappeared during convalescence. The other case, examined during the febrile stage showed normal figures. It seem probable that pyelitis in the absence of marked involvement of the kidney proper is not accompanied by azotemia.

C. Stricture of the Urethra, with Ascending Infection.—Two cases were encountered in this group. Both showed marked retention. The first patient (No. 32) suffered from extravasation of urine, the result of gangrenous cystitis with perforation of the bladder. The second (No. 33), a man with putrid hemorrhagic cystitis, the result of stricture, died with symptoms of uremia of the asthenic type. The blood nitrogen was excessively increased, measuring 209 mg.

D. Enlargement of the Prostate with Retention.—Two cases of simple hypertrophy of the prostate with retention of urine showed a considerable increase of the blood nitrogen, up to 50 mg. One patient (No. 35) was operated on immediately and died five days later. The other was put on a low protein diet for five days, at the end of which time the nitrogen had fallen to normal. Prostatectomy was then performed and the patient made a good recovery. In a case of cancer of the bladder (Case 38) in which the nitrogen rose in five days from 58 to 99 mg., the patient was operated on soon after the second blood examination and died four days afterward. The cases are too few to draw conclusions from, but it seems probable that patients with a high blood nitrogen are poor operative risks and that better results will be

—Lead Poisoning

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Few hyaline, granular casts; R.B.C.; W. B. C.	Liquid	35.5	25.1	First attack.
Many hyaline casts; W. B. C.	Liquid	38.4	29.2	Attacks 2 years and again 1 week ago.
Occasional hyaline; rare R. B. C. W. B. C.	Mixed	42.2	23.4	Colic off and on for 2 years.
Few hyaline casts; W. B. C.	Soft	47.7	32.5	Drop wrist. Similar attack 3 years ago.
Many hyaline, granular casts; R. B. C.; W. B. C.	Soft	51.5	29.2	First attack. Exposed to lead many years.
Hyaline, granular casts	Liquid	54.0	....	First attack 1 year ago.
....	Liquid	99.0	71.6	Painter. Colic 20 years ago and last year. Delirium and uremic coma. Autopsy: small granular kidneys.

attained by reducing the nitrogen by means of a diet poor in protein before operation.

III. LEAD-POISONING

The deleterious action of lead on the kidneys is clearly shown by the figures in Table 4. In every case there is a definite increase in nitrogen and urea, even in those entering hospital with their first attack of colic. All degrees of retention of waste products are seen, up to uremia (Case 44), the final event in so many cases of lead-poisoning.

TABLE 5.—Complications—

Case No.	Age	Diagnosis	Duration of Pregnancy Mos.	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
45	35	Eclampsia	6	128	18	340+	1.028	V. S. T.
46	21	Eclampsia*............		118	65	680	1.020	V. S. T.
47	26	Eclampsia	7	220	30	S. S.	1.028	S. T.
48	28	Eclampsia	9	...	..	S. S.	1.024	H. T.
49	35	Eclampsia, threatened.	4	124	25	420	1.023	S. T.
50	22	Nephritis of pregnancy	6	151	..	620	1.030	0.5%
51	25	Nephritis of pregnancy	7½	170	.. 59	350	1.032	1.2%
52	29	Hyperemesis gravidarum	4	145	36	385	1.027	V. S. T.
53	30	Hyperemesis gravidarum	4	...	..	S. S.	1.022	T.

* Postpartum.

IV. THE COMPLICATIONS OF PREGNANCY

Farr and Williams,[16] working with Folin's methods, have shown that the figures in normal pregnancy are practically the same as in the non-pregnant.

The eclampsia of pregnancy has often been compared with uremia, and even considered identical with it. The results of blood analysis, however, show that in a large proportion of the cases there is little or no retention of nitrogenous waste products in eclampsia, while in uremia there is almost invariably a very marked retention. Thus all of our four cases (Table 5) yielded figures for nitrogen below 40 mg., while six out of the seven cases of Farr and Williams were below 55 mg.; the seventh had 72 mg. In uremia we have found figures of 100 mg. or over in all but one of our cases.

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[16] Farr, C. B., and Williams, P. F.: Am. Jour. Med. Sc., 1914, cxlvii, 556.

This striking difference between the two conditions is of importance from the point of view of pathogenesis, indicating that eclampsia and uremia are quite distinct from one another. It is also of great diagnostic value. The differential diagnosis of eclampsia from uremia has been up to the present in a highly unsatisfactory state, but with the aid of blood analysis it should now be possible with a high degree of probability.

—of Pregnancy

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Many hyaline, granular casts	Milk	25.0	14.1	20 convulsions in last 48 hours. Developed acute mania. Recovered. Did not miscarry.
Few hyaline casts	Liquid	29.5	18.5	Three convulsions to-day. Recovered.
Many hyaline, granular casts; R. B. C.; W. B. C.	None	32.2	14.8	Fifty convulsions in past 24 hours. Comatose. Recovered.
Hyaline, granular casts; W. B. C.	None	39.7	19.9	Typical case. Died 12 hours later.
Occasional R.B.C.; W. B. C.	Mixed	23.1	11.5	Para-7. Eclampsia with fifth pregnancy. Induced labor for threatened eclampsia with sixth pregnancy. Headache; scanty urine. Discharged relieved.
Hyaline, granular casts; W. B. C.	Soft	28.9	14.1	Headache; edema. Discharged relieved.
Hyaline, granular casts; W. B. C.	Liquid	39.7 24.7	21.4 12.6	First blood analysis, 4/15/14. Delivered, 4/18/14. Second analysis, 4/29/14. Recovered.
Hyaline casts; W. B. C.	Soft	20.7	8.0	Vomiting for 9 weeks. Recovered.
Hyaline, granular casts	None	28.7	13.7	Vomiting for 5 weeks. Died same day.

In the peculiar condition known as the nephritis of pregnancy, we have found nearly normal values in two cases. Farr and Williams report three cases, of which two were practically normal; the third showed 52 mg. of nitrogen, falling to normal after delivery.

In hyperemesis we have found normal figures in two cases, one of them fatal, and Farr and Williams examined one case with the same result.

V. ACUTE INTESTINAL OBSTRUCTION

In this condition we have found a remarkable increase in the nonprotein nitrogen and urea, as marked as in most cases of uremia. In one of the three cases the obstruction was mechanical, due to a band; in the other two it was of the paralytic variety. In a case of simple constipation examined for the sake of comparison, there was no retention, although the bowels had not moved for three days (Case 57). The complete disappearance of the retention in the two cases ending in recovery is evidence that the azotemia was not due to complicating disease of the kidneys.

TABLE 6.—Acute—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
54	F	70	Strangulated hernia	...	25 43	730	1.020	V. S. T.
55	F	26	Acute obstruction	116	..	500+	1.011	S. P. T.
56	M	60	Paralytic ileus, following prostatectomy	140	70	S. S.	1.028	S. T.
57	F	21	Simple constipation	124	77	380+	1.022	S. P. T.

The case of mechanical obstruction (No. 55) showed on the day of operation 92 mg. of nitrogen, and nine days later, although the obstruction had been relieved, 169 mg.; death took place the following day. The postoperative symptoms in this case were delirium, vomiting, apathy and profound asthenia, symptoms strongly suggestive of uremia. No necropsy was permitted.

The two cases of paralytic ileus are very instructive, for they show that it is possible to recover from a very high degree of retention, provided that it is not too prolonged. In Case 56 the symptoms of acute intestinal obstruction developed two days after the operation of prostatectomy by the perineal route. Blood analysis the following day revealed 150 mg. of nitrogen and 92 mg. of urea. In spite of the alarming condition, recovery occurred, and the figures for nitrogen and urea fell to normal.

In the other case of paralytic ileus (No. 54) following an operation for strangulated hernia, the blood was taken after improvement had begun, and showed 76 mg. of nitrogen, falling to normal later; it is probable the retention would have been more marked if the blood had been examined earlier.

The observation of a high degree of retention in acute intestinal obstruction is new, so far as we are aware. It suggests that some of the symptoms, such as profound asthenia and apathy, may be due to the retention of waste products.

In order to explain this striking phenomenon, it is necessary to recall the pathologic physiology of this condition.

—Intestinal Obstruction

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Occasional hyaline casts; few R.B.C.; W. B. C.	Liquid Mixed	76.1 29.2	45.5 15.2	Operation, 4/6/14. Paralytic ileus, 4/10/14. Blood taken 3 days later, after improvement had begun. Recovered. Second analysis 2 weeks after first.
Few granular casts; W. B. C.	Liquid	92.2 169.0	72.8 114.0	Acute obstruction due to band, 3 days duration. Relieved by operation, 4/1/14, date of first blood analysis. Mental confusion, apathy, but no convulsions or coma. Second analysis, 4/10/14. Died, 4/11/14.
Few hyaline, granular casts; many W. B. C.	Liquid	150.3 27.6	92.0 15.4	Prostatectomy, 4/9/14, followed by paralytic ileus lasting several days. First blood analysis, 4/12/14; second, 5/1/14. Recovered.
Occasional hyaline cast; W. B. C.	Mixed	30.4	13.9	Duration, 3 days.

There is an enormous increase in the secretion into the intestine, and owing to the stagnation of the intestinal contents, a marked increase in the decomposition of nitrogenous substances, as shown by the large amounts of indican in the urine. There is therefore an opportunity for increased absorption of non-protein nitrogen from the intestine. The profound shock and the possible intoxication by poisonous substances absorbed from the intestine (cf. Whipple[17]) must affect unfavorably the renal function, leading to defective elimination of waste products. And, lastly, the great loss of fluid by vomiting,[18] with the resulting concentration of the blood, is undoubtedly a factor, though not enough in itself to account for a five-fold increase in the total non-protein nitrogen.

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[17] Whipple, Stone and Bernheim: Jour. Exper. Med., 1913, xvii, 286.

[18] Hartwell, John A., Hoguet, J. P., and Beekman, Fenwick: An Experimental Study of Intestinal Obstruction, The Archives Int. Med., 1914, xiii, 701.

VI. DISEASES OF THE HEART AND AORTA

Three cases of pericarditis with effusion and a case of severe acute endocarditis ending in recovery, all showed normal figures, as did also a compensated case of extensive mitral and aortic valvular disease, and a patient with aortic aneurysm. A typical case of subacute infective endocarditis (Case 63) with anemia and enormous spleen (1035 gm.) showed considerable retention. At the necropsy the kidneys showed recent infarcts and multiple pin-point-sized hemorrhages. Microscopically, there were the signs of an acute diffuse nephritis, and many glomeruli showed hemorrhages into the capsular space. One miliary abscess was seen.

TABLE 7.—DISEASES OF—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
58	M	8	Pericarditis with effusion, rheumatic	88	80	270	1.022	S. P. T.
59	M	17	Pericarditis with effusion, rheumatic	140	68	740	1.028	S. P. T.
60	M	38	Pericarditis with effusion, tuberculous	110	37	1,280	1.018	V. S. T.
61	F	23	Endocarditis, chronic....	127	63	S. S.	1.019	S. P. T.
62	F	19	Endocarditis, acute.......	146	54	350+	1.019	S. T.
63	M	22	Endocarditis, malignant.	115	45	925	1.011	S. T.
64	M	55	Aneurysm of aorta......	140	70	600	1.023	V. S. T.

The results in cardiac decompensation have been stated already under the heading "chronic passive hyperemia of the kidneys."

VII. THE ACUTE INFECTIONS

A. Pneumonia.—In acute lobar pneumonia we have found a moderate degree of retention in the majority of the cases, nine out of fourteen patients showing more than 35 mg. of nitrogen; the highest figure was 50 mg. The retention reached its maximum toward the crisis and disappeared early in convalescence. It was not dependent on the absorption of the exudate, for it occurred before resolution took place. The figures were lower in the younger patients, three out of the four under 20 years of age showing normal values. There was no apparent relation between the degree of retention and prognosis.

B. Typhoid Fever.—In typhoid we have met normal or slightly elevated figures in the small series studied (five cases). This is in agreement with the infrequency of severe lesions of the kidney in typhoid.

—the Heart and Aorta

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Few hyaline casts; W. B. C.	Soft	22.7	11.0	Chronic mitral disease. Recovered.
W. B. C.	Soft	23.2	11.5	Chronic mitral and aortic disease. Developed pleural effusion and pneumonia later. Recovered.
Occasional hyaline cast; R. B. C.; many W. B. C.	Soft	29.2	15.7	Lymphocytosis in pericardial fluid. Tapped several times.
Hyaline casts; few R. B. C.; many W. B. C.	Mixed	30.2	14.8	Mitral and aortic stenosis and insufficiency, compensated. Rheumatic.
Occasional hyaline cast; R. B. C.; W. B. C.	Mixed	30.2	16.1	Acute aortic endocarditis, with fever lasting 7 weeks. Recovered.
Few hyaline, granular, pus casts; R. B. C.; W. B. C.	Soft	68.0	42.5	Typical subacute infective endocarditis. At necropsy, kidneys showed recent infarcts and acute diffuse nephritis.
Occasional hyaline cast; W. B. C.	Soft	30.9	16.1	No failure of compensation.

C. Acute Articular Rheumatism.—This has yielded very low figures, probably because of the small amount of nourishment taken by these patients and the lack of renal involvement.

D. Scarlet Fever.—In scarlatina, contrary to our expectations, there was not the slightest retention in any of the four cases studied. None of these, to be sure, was very severe.

VIII. SYPHILIS

We have examined eleven cases of this disease, two in the primary, four in the secondary and five in the tertiary stage. In every case in which the urine was examined, albumin and casts were found.

Of the primary cases, one was normal, the other moderately elevated (36 mg.).

TABLE 8.—Acute—

Case No.	Sex	Age	Diagnosis	Day of Disease	Systolic Blood Pressure mm. Hg	Urine			
						Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
65	M	17	Pneumonia, acute lobar	5	140	..	S. S.	1.023	0.1%
66	M	9	Pneumonia, acute lobar	4	85	68	365	1.024	S. P. T.
				7	...	..	....	.....	.....
67	F	16	Pneumonia, acute lobar	5	125	35	750	1.026	0.05%
				7	...	..	....	.....	.....
				9	...	..	....	.....	.....
68	M	33	Pneumonia, acute lobar	..	...	50	450	1.017	S. P. T.
69	M	27	Pneumonia, acute lobar	6	...	80	1,520	1.016	S. P. T.
70	M	36	Pneumonia, acute lobar	8	108	30	670	1.024	S. T.
				16	...	..	....	.....	.....
71	M	28	Pneumonia, acute lobar	4	135	..	770	1.024	S. P. T.
72	M	39	Pneumonia, acute lobar	4	108	..	465+	1.026	S. P. T.
73	M	39	Pneumonia, acute lobar	6	85	..	450	1.017	S. P. T.
74	M	25	Pneumonia, acute lobar	8	120	60	850	1.026	S.P.T.
				15	...	..	....	.....	.....
75	M	51	Pneumonia, acute lobar	8?	140	..	1,730	1.016	S. T.
76	M	48	Pneumonia, acute lobar	13?	160	..	680	1.020	S. P. T.
				16?	...	..	....	.....	.....
77	M	15	Pneumonia, acute lobar	..	88	80	385	1.022	V.S.T.
78	F	40	Pneumonia, acute lobar	10	102	41	S. S.	1.020	V.S.T.
				12	...	..	....	.....	.....
79	F	18	Typhoid fever	16	100	61	730	1.016	S. P. T.
80	M	15	Typhoid fever	19	105	..	730	1.021	S. P. T.
						62	....	.....	.....
81	M	36	Typhoid fever	17	105	67	1,040	1.017	V.S.T.
82	F	10	Typhoid fever	12	115	71	270	1.020	V.S.T.
83	M	30	Typhoid fever	20	90	..	S.S.	1.025	0.05%
84	F	23	Acute articular rheumatism	3	110	..	700	1.020	S. P. T.
85	M	28	Acute articular rheumatism	16	112	70	640	1.026	S. P. T.
86	F	23	Erythema nodosum	21	100	..	575	1.020	S. P. T.
87	F	19	Puerperal sepsis	..	102	50	350	1.013	0.15%
88	M	19	Scarlatina	5	...	..	S. S.	1.025	S. P. T.
89	F	10	Scarlatina	4	...	..	870	1.018	S. P. T.
90	M	23	Scarlatina	4	...	..	800	1.025	S. P. T.
91	M	14	Scarlatina	4	...	..	825	1.018	S. P. T.
92	M	19	Measles	6	...	45	1,670	1.020	S. P. T.
93	M	18	Meningococcus meningitis	..	138	50	835	1.021	S. P. T.
94	M	23	Appendicitis with retrocecal abscess	2	115	..	S. S.	1.025	0.0

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* The typhoid diet employed is a generous one of about 3,000 calories, containing

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Many brown granular casts; R. B. C.; W. B. C.	Liquid	23.2	11.2	Died on seventh day.
W. B. C.	Soft	23.1 24.0	11.8 12.6	Crisis ended next day. Moderately severe case. Recovered.
Few hyaline, granular casts	Liquid	27.0 23.0 28.5	14.0 13.4 13.4	First normal temperature on ninth day. Severe case. Recovered.
Few granular casts; W. B. C.	Soft	28.1	14.3	Died 2 days later.
Occasional hyaline cast; W. B. C.	Soft	30.0	16.6	Recovered.
Granular casts; W. B. C.	Soft	31.7 40.5	.... 27.6	Acute followed by chronic pneumonia and failure of resolution. Recovered.
W. B. C.	Soft	33.5	18.4	Crisis next day. Recovered.
Many hyaline casts; W. B. C.	Soft	36.2	19.1	Recovered.
Many granular casts; R. B. C.; W. B. C.	Soft	39.2	30.3	Died 4 days later.
Hyaline, granular casts R. B. C.; W. B. C.	Soft	40.2 32.4	26.9 19.0	 Recovered.
Hyaline, granular casts; W. B. C.	Soft	41.0	27.8	Died 13 days later.
Granular casts; W. B. C.	Soft	41.5 44.0	24.1 31.7	Died 6 days after first blood analysis.
Hyaline, granular casts; W. B. C.	Soft	43.7 50.0 31.2	30.9 36.6 19.6	3/6/14. 3/9/14. After crisis. 3/13/14. Crisis 2 days after first analysis. Recovered.
Hyaline, granular casts; W. B. C.	Liquid	46.2 44.0	30.0 25.3	Complicated with jaundice. Crisis on eleventh day. Recovered.
No casts; few R. B. C.; W. B. C.	Soft	21.1	10.1	Temp. 103.8 F. Moderately severe.
Rare hyaline cast; R. B. C.; W. B. C.	Typhoid*	27.0 25.6	13.7 12.9	4/ 4/14. Temp. 104 F. 4/27/14. Temp. 100 F. Moderately severe.
Occasional hyaline cast; few R.B.C.; many W. B. C.	Soft; forced liq.	27.2	13.3	Temp. 102 F. Moderately severe.
Few hyaline casts; few R.B.C.; many W. B. C.	Typhoid*	29.2	15.8	Temp. 103 F. Moderately severe.
Many hyaline, granular casts	Liquid	33.5	19.2	Temp. 103 F. Died on thirty-ninth day of disease.
W. B. C.	Soft	19.6	9.4	Moderately severe.
Hyaline casts; rare R. B. C.; W. B. C.	Soft	27.4	15.4	Moderately severe.
Many W. B. C.	Liquid	22.7	11.5	Prolonged fever.
Pus	Soft, with meat once daily	22.5	11.1	Recovered.
Occasional hyaline cast; R. B. C.	Moderate protein.†	21.2	11.7	Mild case.
Negative	Soft	23.2	10.9	Moderately severe.
W. B. C.	Mixed	27.7	13.8	Moderately severe.
Negative	Soft	29.1	14.1	Mild case.
Negative	Mixed	27.4	18.4	
R. B. C.; W. B. C.	Soft	27.3	15.6	Severe case. Treated with serum. Temp. 104 F. on day of analysis. Recovered.
No casts; few R. B. C.; many W. B. C.	Soft	29.7	18.0	

100 gm. protein. † No meat or fish.

The majority of those in the secondary stage was also normal, only one showing an increase (41 mg.).

In the tertiary stage there was more evidence of retention, one case showing 51 mg., another 55 mg. of nitrogen, while the remaining three were normal.

For the whole series we find 36 per cent. with a total non-protein nitrogen of 35 mg. or more. Folin and Denis,[19] in a much larger series of 63 cases, reported 46 per cent. showing 35 mg. or more; their highest figure was 45 mg. Their patients were in all stages of the disease; no clinical details are given.

TABLE 9.—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
95	M	26	Primary	102	64	1,150	1.013	V. S. T.
96	M	34	Primary	...	70	1,300	1.017	V. S. T.
97	M	28	Secondary	...	..	.....	.....	.....
98	F	38	Secondary	...	..	.....	.....	.....
99	F	24	Secondary	...	..	.....	.....	.....
100	M	47	Secondary, early	...	..	.....	.....	.....
101	M	46	Tertiary	130	..	460	1.026	V. S. T.
102	M	42	Tertiary	160	60	910	1.030	0.2%
103	F	45	Tertiary	110	..	(?) 220	1.023	S. P. T.
104	M	28	Tertiary	...	..	S. S.	1.015	S. P. T.
105	M	53	Tertiary	...	..	S. S.	1.024	S. P. T.

From these data it seems fair to conclude that the kidneys are affected in a very considerable proportion of cases in all stages of the disease. This involvement is probably temporary as a rule, but may often go on to permanent changes in the kidney, usually of the interstitial type. Further investigation along these lines is desirable. The effect of antisyphilitic treatment, especially salvarsan, should be studied.

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[19] Folin, O., and Denis, W.: Jour. Biol. Chem., 1913, xiv, 29.

IX. TUBERCULOSIS

Out of three cases of pulmonary tuberculosis, all advanced and active, two showed a considerable retention. The study of a large series should yield interesting results.

X. DISEASES OF THE NERVOUS SYSTEM

Cerebral hemorrhage (including one case of skull fracture) has not been accompanied by any notable azotemia. It is thus possible to distinguish between apopleptic and uremic coma by means of blood analysis.

—Syphilis

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
....	Mixed	29.5	14.9	Chancre 3 weeks ago. Wassermann ++. Untreated.
Hyaline, fine granular casts; W.B.C.	Mixed	36.0	19.6	One dose of neosalvarsan, 16 days ago.
....	Mixed	23.7	14.0	Chancre 1 year ago. Now mucous patches in mouth. Untreated.
....	Mixed	24.1	10.4	Sore throat for 2 months. Mucous patches on tongue and tonsils; spirochetes in same. Untreated.
....	Mixed, low	26.8	15.1	Flat papular syphilid, generalized. Salvarsan 6 days ago.
....	Mixed	41.0	21.3	Chancre on chin. Roseola. Untreated.
Few granular casts	Soft	25.7	12.8	Epileptiform seizures, followed by paralysis of arm. Blood and spinal Wassermann negative.
Granular casts; W. B. C.	Purin free	28.0	....	1/24/14. Aneurysm of aorta; nephrolithiasis. Wassermann +.
		26.2	13.7	2/ 4/14.
Rare hyaline cast; W. B. C.	Mixed	28.5	14.0	Obstinate headaches; relieved later by salvarsan.
Hyaline, granular casts; R. B. C. W. B. C.	Mixed	51.2	28.1	Periostitis. Infection 14 years ago.
Hyaline, granular casts; W. B. C.	Mixed, little meat	55.0	35.9	Extensive ulceration of soft palate. Duration 1 year. Liver and spleen both enlarged.

Neurasthenia and hysteria have shown normal values, as was to be expected. The three cases of hysteria were all studied immediately after an attack of convulsions.

XI. MISCELLANEOUS DISEASES

A. The Anemias.—Three of the four cases of anemia have shown a considerable degree of azotemia. In two of these hemolysis was very evident, and the retention may perhaps be explained by the clogging of the tubules of the kidney with hemoglobin and disintegrated red cells, such as has been demonstrated in fatal cases of "black-water fever." This would apply especially to Case 121, in which hemoglobinuria and reduction of the red count to one million occurred in a case of atypical lobar pneumonia due to the pneumococcus. In the other case of hemolytic anemia (No. 122) a woman with one million red cells, slight jaundice and an enlarged spleen, the nitrogen reached the very high figure of 109 mg., and the phenolsulphonephthalein output was nil. There was stupor, but no other sign of uremia. With improvement in the blood condition the nitrogen content returned to normal, and the phenolsulphonephthalein excretion rose to 31 per cent.

A case of polycythemia showed 44 mg. of nitrogen.

TABLE 10.—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
106	F	32	Pulmonary	140	50	600+	1.020	S. P. T.
107	M	49	Pulmonary	133	63	1,220	1.020	S. P. T.
108	M	21	Pulmonary	100	..	560	1.027	S. P. T

B. Diabetes.—Uncomplicated cases have shown no retention. Two typical cases of diabetic coma have shown a considerable azotemia, up to 67 mg. of nitrogen, which is not surprising in such a grave disturbance of metabolism. A third case, however, with deep breathing and on the verge of coma, showed normal figures (Case 126).

C. Gout.—Both cases had a moderate retention, which can be referred to involvement of the kidneys in the gouty process.

D. Myxedema.—Each of the two cases of myxedema was complicated with chronic nephritis. Curiously enough, the one with very high blood-pressure showed normal values, while the other, with normal blood-pressure, had a marked azotemia (65 mg. nitrogen). This is a good illustration of the absolute lack of correspondence between the blood-pressure and the non-protein nitrogen. Folin and Seymour[20] have called attention to this point.

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20. Folin, O., Denis, W., and Seymour, M.: The Non-Protein Nitrogenous Constituents of the Blood in Chronic Vascular Nephritis (Arteriosclerosis) as Influenced by the Level of Protein Metabolism, The Archives Int. Med., 1914, xiii, 224.

The influence of thyroid secretion on metabolism is shown by the considerable rise in non-protein nitrogen and urea in both these cases when thyroid extract was administered. The increase averaged 6.1 mg. for the nitrogen and 4.5 mg. for the urea.

E. Exophthalmic Goiter.—Normal values were found in one case, and also in another complicated with diabetes (Case 128).

F. Malignant Disease.—One of the three cases of carcinoma showed a considerable retention; the other two were normal.

G. Acute Yellow Atrophy of the Liver.—We have examined one case of this disease (No. 137), typical in all respects except in the termination in recovery. During the height of the symptoms there was a considerable retention of waste nitrogen. The chief point of interest, however, lies in the urea, which at the first two examinations was decidedly below the normal, constituting 32 per cent. and 41 per cent. of the total nitrogen, respectively, while usually with retention there is an increase in the urea fraction, often up to 70 or 80 per cent. of the whole. This phenomenon is in accord with the diminished excretion of urea, and the supposed diminished urea formation in the liver in this disease. The last analysis, made as the patient was beginning to improve, though still deeply jaundiced, showed nearly normal figures.

—Tuberculosis

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Few hyaline casts; R. B. C.; W. B. C.	Mixed	22.9	12.7	Temp. 101 F. Third stage.
Hyaline, granular casts; R. B. C.; W. B. C.	Soft	40.0	26.2	Febrile. Second stage. Profuse hemorrhage 11 days previous to analysis.
Occasional hyaline cast; W. B. C.	Soft	43.5	19.9	Febrile. Second stage.

The Effect of Diet in Azotemia.—Folin, Denis and Seymour[20] have investigated the effect of a protein-poor diet in chronic interstitial nephritis, and found in every case a marked reduction in both nitrogen and urea, usually though not always to normal or below. Their highest figure for nitrogen was 58 mg. We also have experimented in this way in a few cases, using the protein-poor diet recommended by Goodall.[21] In two cases (Nos. 12 and 36) a considerable retention of about 50 mg. nitrogen was caused to disappear by diet. In uremic patients with excessively high figures (cf. Case 16) we have not been able to reduce the azotemia to any important degree.

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[21] Goodall, H. W.: Boston Med. and Surg. Jour., 1913, clxviii. 760.

Prognosis and Blood Analysis.—In chronic nephritis and obstruction of the urinary tract resulting from urethral stricture and cancer of the bladder, we have found that a nitrogen value of over 90 mg. has been followed by death within a period of thirty-five days in all but one of ten cases. The exception was Case 13, previously referred to. Four patients with over 200 mg. of nitrogen all died in nineteen days or less. When, however, the condition leading to excessive retention is temporary, as, for instance, in acute intestinal obstruction (Case 56), or hemolytic anemia (Case 122), a total nitrogen value of even 150 mg. is not inconsistent with recovery.

TABLE 11.—Diseases—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
109	M	63	Fracture of skull with cerebral hemorrhage	180	30	S. S.	1.025	V. S. T.
110	M	60	Cerebral hemorrhage	205	59	S. S.	1.030	S. P. T.
111	F	53	Cerebral hemorrhage	220	50	S. S.	1.027	0.2%
112	M	52	Cerebral hemorrhage	190	..	S. S.	1.020	H. T.
113	F	43	Neurasthenia	140	..	S. S.	.....	S. P. T.
114	M	54	Neurasthenia	140	..	S. S.	1.022	S. P. T.
115	F	48	Neurasthenia	140	..	S. S.	1.014	S. P. T.
116	F	28	Hysteria	...	..	S. S.	1.012	T.
117	F	38	Hysteria.................	...	..	S. S.	1.004	S. P. T.
118	F	30	Hysteria	135	50	550	1.023	S. P. T.

SUMMARY AND CONCLUSIONS

Analyses of the blood for total non-protein nitrogen and urea nitrogen have been made by the methods of Folin in five healthy adults and 142 hospital cases.

1. In the fasting healthy adult the total non-protein nitrogen varied betweeen 22.9 and 25 mg. per 100 c.c. of blood, and the urea nitrogen between 12 and 14 mg.

2. The effect of a full meal with meat in the case of the healthy adult was a rise of total non-protein nitrogen averaging 4.7 mg., and of urea, averaging 2.5 mg.

3. In both chronic interstitial and chronic diffuse nephritis the cases without symptoms of uremia showed normal or moderately elevated values; the uremic cases, with one possible exception, showed a great increase in both nitrogen and urea.

4. The excretion of phenolsulphonephthalein was roughly proportionate to the degree of retention; the cases with 100 mg. or over of total nitrogen all showed 5 per cent. or less phenolsulphonephthalein excretion. Many cases, however, with a considerable impairment of phenolsulphonephthalein excretion showed no signs of retention, and a moderate amount of retention of waste nitrogen often occurred with no impairment of the elimination of phthalein.

—OF THE NERVOUS SYSTEM

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
R. B. C.; W. B. C.	None	20.2	10.0	Convulsions. Fatal case.
Few hyaline, granular casts	Soft	27.0	13.7	Recovered.
Hyaline, granular casts; W. B. C.	Liquid	28.7	14.1	Died 45 days later.
Hyaline, granular casts	....	36.1	18.6	Died few hours later.
No casts	Mixed	22.7	11.5	
No casts	Mixed	25.7		
No casts	Mixed	27.8	13.5	
Numerous hyaline, granular casts; W. B. C.	Mixed	22.0	12.0	Hysterical convulsions.
No casts	Mixed	26.5	14.4	Hysterical convulsions.
Hyaline casts; W. B. C.	Soft	27.2	12.0	Hysterical convulsions.

5. The proportion of urea nitrogen to the total non-protein nitrogen in disease varied from 32 per cent. to 85 per cent. Where the nitrogen was normal the urea usually was about one-half the total nitrogen; where it was elevated, the urea usually but by no means always constituted about 70 per cent. of the whole. No reasons could be found for these variations. The determination of the total non-protein nitrogen alone is therefore more valuable than that of the urea alone.

TABLE 12.—Miscellaneous—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
119	F	39	Anemia, secondary	115	66	640	1.020	V. S. T.
120	M	28	Anemia, secondary	125	69	760	1.022	V. S. T.
121	M	45	Anemia, hemolytic	135	..	2,640	1.012	0.2%
122	F	25	Anemia, hemolytic	120	0	700	1.020	S. T.
					31	820	1.020	S. P. T.
123	M	18	Anemia, Banti's disease..	...	33	S. S.	1.015	S. P. T.
124	M	43	Polycythemia	130	..	1,530	1.012	S. P. T.
125	F	59	Diabetes	155	80	.....	.....	.....
126	M	17	Diabetes	95	63	5,370	1.017	V. S. T.
127	F	55	Diabetes	165	80	1,500	1.023	S. P. T.
128	F	37	Diabetes, with exophthalmic goiter	...	75	1,840	1.019	S. P. T.
129	M	64	Diabetes, with anemia...	145	47	1,360	1.020	S. P. T.
130	M	40†	Diabetes, with coma.....	...	10	1,220+	1.024	S. P. T.
131	M	44	Diabetes, with coma.....	...	..	S. S.	.....	S. T.
132	M	50	Gout	150	..	500	1.030	S. P. T.
133	M	73	Gout	117	60	1,080	1.021	S. P. T.
134	F	66	Myxedema	215	58	1,640	1.010	S. P. T.
135	F	61	Myxedema	120	30	1,280	1.014	S. P. T.
136	F	36	Exophthalmic goiter	128	65	700	1.023	S. P. T.
137	M	23	Acute yellow atrophy....	120	..	S. S.	1.026	V. S. T.
138	F	43	Cirrhosis of liver........	...	..	S. S.	1.012	V. S. T.
139	M	49	Carcinoma of liver, primary	...	..	400+	1.022	V. S. T.
140	M	65	Carcinoma of pancreas...	130	..	S. S.	1.018	S. T.
141	M	66	Carcinoma of pancreas...	115	..	500	1.021	S. T.

† About.

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Occasional hyaline cast; few R.B.C.; W. B. C.	Mixed	30.4	14.8	Hb., 10-15 %; R. B. C., 2,160,000; nucleated reds. Uterine hemorrhage following abortion.
W. B. C.	Soft	50.0	28.1	Hb., 15%; R. B. C., 1,460,000; nucleated reds. Profuse hemorrhage from stomach and intestine shortly before analysis, duodenal ulcer.
Numerous hemaglobin casts; rare R. B. C.	Liquid	83.7	59.7	Atypical acute lobar pneumonia, complicated with hemoglobinuria setting in on twelfth day, and lasting 3 days. Blood analysis on fifteenth day. Red count, 1,072,000. Died on twenty-first day.
Few hyaline casts; few R. B. C.; W. B. C.	Soft	108.8	79.0	6/26/14 Hb., 20%; R.B.C., 780,000; W.B.C. 32,000.
Few hyaline casts; few R. B. C.; W. B. C.	Mixed	26.1	....	7/17/14 Hb., 60%; R.B.C., 2,820,000; W.B.C. 6,200. At first analysis, nucleated reds; increased fragility of red cells; enlarged spleen and slight jaundice.
Few hyaline casts; W. B. C.	Refused food	39.7	21.3	Hb., 35%; R.B.C., 2,216,000. Died 2 days later.
No casts; occasional R. B. C.	Mixed	43.7	24.3	R.B.C., 7,900,000. Enlarged spleen with cyanosis.
....	Janeway	21.1	13.6	Sugar, 2.3%. Moderately severe case.
Hyaline, granular casts; R. B. C.	Janeway	25.0	9.0	Acidosis with deep breathing. Died in coma 4 days later.
Occasional hyaline cast; W. B. C.	Janeway	27.5	16.7	Sugar, 5.0% on entrance; 0.5% on day of blood analysis. Moderately severe case.
Hyaline, granular casts; R. B. C.; W. B. C.	Janeway	27.7	13.7	Sugar, 4.5% on entrance; 0.7% on day of analysis. Developed acidosis 2 days later and died suddenly the same day, not in coma.
Rare hyaline cast; W. B. C.	Janeway	29.4	13.8	Probably pancreatic diabetes. Blood-count: Hb., 35%; R.B.C., 2,400,000; no nucleated reds.
Hyaline, granular casts; R. B. C.; W. B. C.	None	45.8	23.3	Typical acid coma. Died 36 hours after blood was taken.
....	None	66.9	44.0	Large amount of sugar. Typical acid coma. Died few hours after blood was taken.
Hyaline casts; W. B. C.	Soft	35.1	21.2	Two attacks a number of years previous. Tophus in ear.
Hyaline, granular casts; R. B. C.	Soft	38.9	27.0	Typical attacks for many years.
Hyaline, granular casts; W. B. C.	Mixed	23.5 31.2	11.5 13.2	Without thyroid extract. With thyroid, 9 days.
Hyaline, granular casts; W B. C.	Mixed	60.2 64.7	39.8 47.0	Without thyroid extract. With thyroid. No renal symptoms.
Hyaline casts; many R. and W. B. C.	Soft	27.7	13.5	Marked symptoms of hyperthyroidism.
Occasional hyaline, granular casts; R. B. C.; W. B. C.	Liquid	30.4 42.0 44.7 44.2 33.1	9.7 17.1 19.5 23.4 15.0	5/22/14 5/24/14 5/25/14 5/26/14 5/27/14 Jaundice with delirium, mania and stupor. Liver much decreased in size. Leucin and tyrosin in urine. Complete recovery with return of liver to normal size.
Rare hyaline cast; occasional W.B.C.	Mixed	21.2	8.8	Large liver and spleen without ascites. Alcoholism. Positive Wassermann.
Rare granular cast	Mixed	27.0	12.9	Died 12 days later. Diagnosis proved by necropsy.
Hyaline, granular casts; W. B. C.	Fat-free	33.5	13.0	Jaundice with dilated gall-bladder, and typical pancreatic stools.
Hyaline, granular casts; W. B. C.	Soft	41.4	22.6	Jaundice. Carcinoma of head of pancreas at necropsy.

6. The estimation of the total non-protein nitrogen is of the greatest value in the diagnosis of uremia. Amounts of 100 mg. or over were encountered in only two conditions besides uremia, namely, acute intestinal obstruction and profound anemia from hemolysis. Only one case of uremia without marked increase in nitrogen was encountered out of a total of eight cases.

7. The determination of the total non-protein nitrogen is a great aid in the prognosis of chronic nephritis. Patients showing over 100 mg., with one exception, did not live more than thirty-five days.

8. The results of blood analysis furnish the best guide as to the diet to be given in nephritis; cases with a considerable retention require a restriction of the protein, and by this means a return to normal figures may be brought about, if the azotemia is not too pronounced. In cases of outspoken uremia, however, no marked reduction of the azotemia has resulted from a protein-poor diet. Nephritis with a normal amount of non-protein nitrogen does not call for any *marked* decrease of protein in the diet.

9. In chronic passive congestion of the kidneys there is little or no retention of nitrogenous waste products.

10. In pyelitis the presence of azotemia probably indicates involvement of the parenchyma of the kidney.

11. A marked elevation of the non-protein nitrogen or urea renders the patient a poor operative risk, and the azotemia should be overcome by diet, if possible, before an operation is attempted, in all cases in which delay is permissible. In hypertrophy of the prostate, for example, a low-protein diet may be combined with drainage of the bladder as a preliminary to operation.

12. Chronic lead-poisoning was accompanied by evidence of retention in all the cases examined.

13. The eclampsia of pregnancy seldom shows a marked increase in non-protein nitrogen and urea. It is therefore distinct from uremia. Analysis of the blood will usually serve to distinguish between uremia and eclampsia.

14. In acute intestinal obstruction a tremendous increase in the nitrogenous waste products was found in all of the three cases examined. A return to normal took place in the two which recovered.

15. Compensated valvular disease of the heart, aortic aneurysm, acute pericarditis with effusion, and acute endocarditis in the absence of disease of the kidneys, all showed normal values.

16. In acute lobar pneumonia a considerable increase was seen in the majority of cases, reaching its maximum toward the crisis, but bearing no relation to the time at which resolution took place. Typhoid

fever, acute rheumatism and uncomplicated scarlatina showed normal figures.

17. Syphilis showed a considerable degree of retention in 36 per cent. of the cases examined, evident in all stages of the disease.

18. In cerebral hemorrhage, hysteria and neurasthenia no increase was found.

19. Severe anemia due to hemolysis showed a marked retention, reaching in one case 100 mg. of nitrogen.

20. In uncomplicated diabetes the values were normal; both cases examined in coma showed retention.

21. The administration of thyroid extract in two cases of myxedema caused an increase in both nitrogen and urea. Both cases were complicated with chronic nephritis.

22. In exophthalmic goiter the blood nitrogen and urea were normal in amount.

23. No changes were met with in malignant disease which could not be ascribed to a complication with renal disease.

24. In a case of acute yellow atrophy the proportion of urea nitrogen to the total non-protein nitrogen was decreased, although there was a considerable degree of azotemia.

In conclusion we take pleasure in acknowledging our indebtedness to Professor Folin, Dr. Henry Lyman and Dr. Richard P. Bell for helpful advice in the laboratory work, and to the staff of the New Haven Hospital for placing the clinical material at our disposal.

424 Temple Street—1193 Chapel Street.

AN EPIDEMIC OF STREPTOCOCCUS SORE THROAT IN JACKSONVILLE, ILL., WHICH WAS TRACED TO THE MILK OF COWS AFFECTED WITH STREPTOCOCCUS MASTITIS *

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CHICAGO

During the past winter there occurred in Jacksonville, Ill., a city of 15,000 people, an unusual number of cases of sore throat. In order to check the spread of the disease, twenty-five physicians volunteered their services for the systematic examination of all schoolchildren. The epidemic began soon after Thanksgiving Day, 1913, and reached its height in the month of December. In January many persons were on the sick list, but comparatively few new cases were reported. Early in February the number of cases of tonsillitis was not much above the average of other winters. At the request of local physicians the State Food Commission made an investigation of the milk-supply, but it reported nothing to indicate a causal relationship between the milk and the epidemic.

Later in January, through the kindness and cooperation of the local physicians and the city health commissioner, we were enabled to study the epidemic from a clinical as well as an etiological point of view.

The plan of procedure was as follows:

1. A clinical investigation of cases of sore throat and the relation to the milk-supply.
2. Inspection of the cows, dairies and distributing plants.
3. Bacteriological examinations of throat swabs from infected individuals and of milk from cows showing signs of garget.

1. *CLINICAL INVESTIGATION*

SYMPTOMATOLOGY

The outbreak was quite abrupt in the last days of November, rapidly reaching a climax December 4 (Chart 1). After a brief recession there occurred a second prolonged rise in the number of cases that persisted for about two weeks. Thereafter the new cases appeared irregularly.

Altogether, 348 cases of sore throat were reported between Nov. 29, 1913, and Feb. 1, 1914.

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* Submitted for publication July 10. 1914.

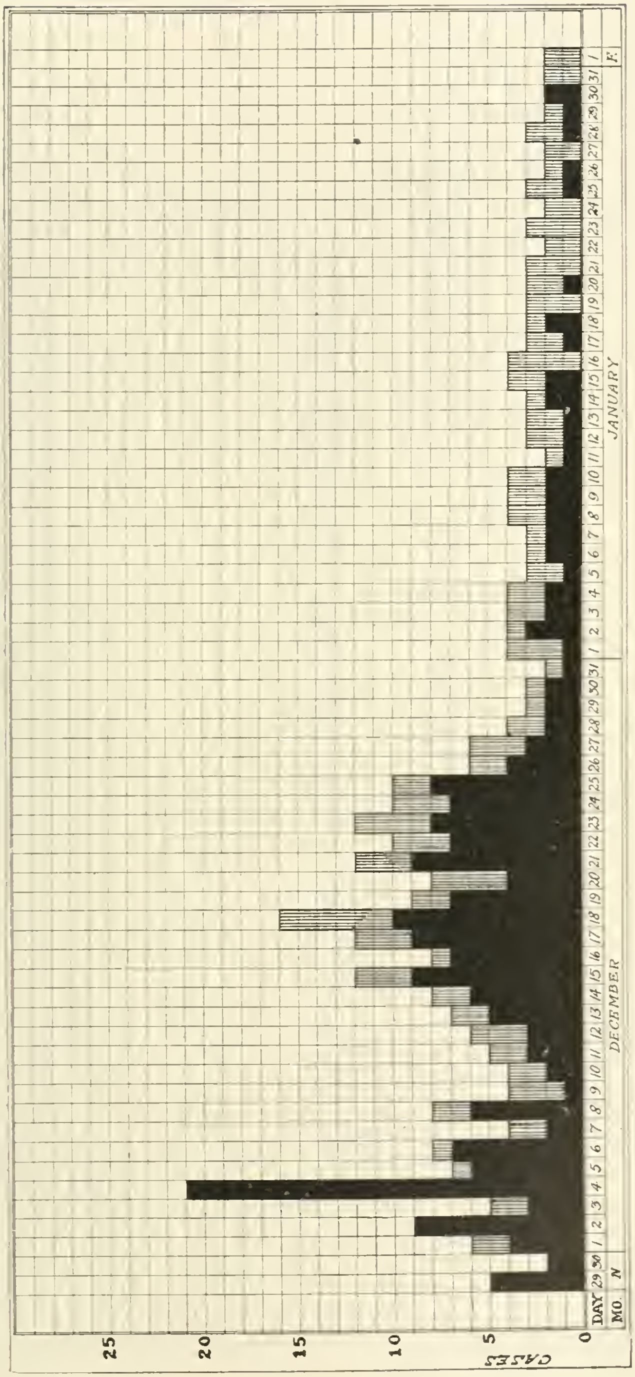

Chart 1.—Outbreak of cases of sore throat by days. Black squares are cases in which milk of Dairy X was used. Lined squares are cases in which milk of other dairies was used.

A definite tonsillitis was described in 217 cases, with appearance of exudate in 104 instances. In addition to a number of cases of true diphtheria with Klebs-Loeffler bacilli, there were 17 cases with an exudate so closely resembling diphtheria that antitoxin was administered. In the latter group Klebs-Loeffler bacilli were not found.

Enlargement of the cervical glands was one of the most constant symptoms, occurring in 192 persons. Among other complications were 5 cases of peritonsillar abscess, 10 of otitis media, 1 of nephritis, and 14 of various skin eruptions, not including 4 cases of erysipelas. Rheumatism of an acute or subacute type was described in 16 cases, and endocarditis in 6.

The duration of the disease gives some idea of its unusual severity and persistence. The average period of illness in nearly 200 cases recorded was between nine and ten days.

Three patients died, two from septicemia and one from peritonitis developing soon after tonsillitis.

Relation of Sore Throat to Milk

(*a*) In a house-to-house canvass carried out by Drs. Thompson and Black, a special inquiry was made concerning the use of milk, cream or ice cream by the sore throat victims and concerning the dairy supplying each household.

In a total of 913 households investigated, 226 histories of sore throat were obtained. In 135 of these cases milk, cream or ice cream was used from a dairy that will be called "Dairy X." Since Dairy X supplied only 160 out of the 913 households, or 17.5 per cent., it is evident that its contribution of 135 out of a total of 226 cases, or 60 per cent., is excessive.

TABLE 1.—Comparative Morbidity and the Milk Supply in the Several Institutions

Institution	Number Residents	Number Cases Sore Throat	Percentage Affected	Dairy
Blind	198	5	2.5	Own
Deaf and dumb	478	34	7.1	Own
Insane	1,725	1	.05	Own
College	200	80	40.0	X
Hospital	50	2	4.0	A

(*b*) There are three large state institutions in Jacksonville, all using milk from their own dairy farms. The physicians in charge furnished us with accurate data on the incidence of sore throat with the approximate time of onset. Similar information was secured

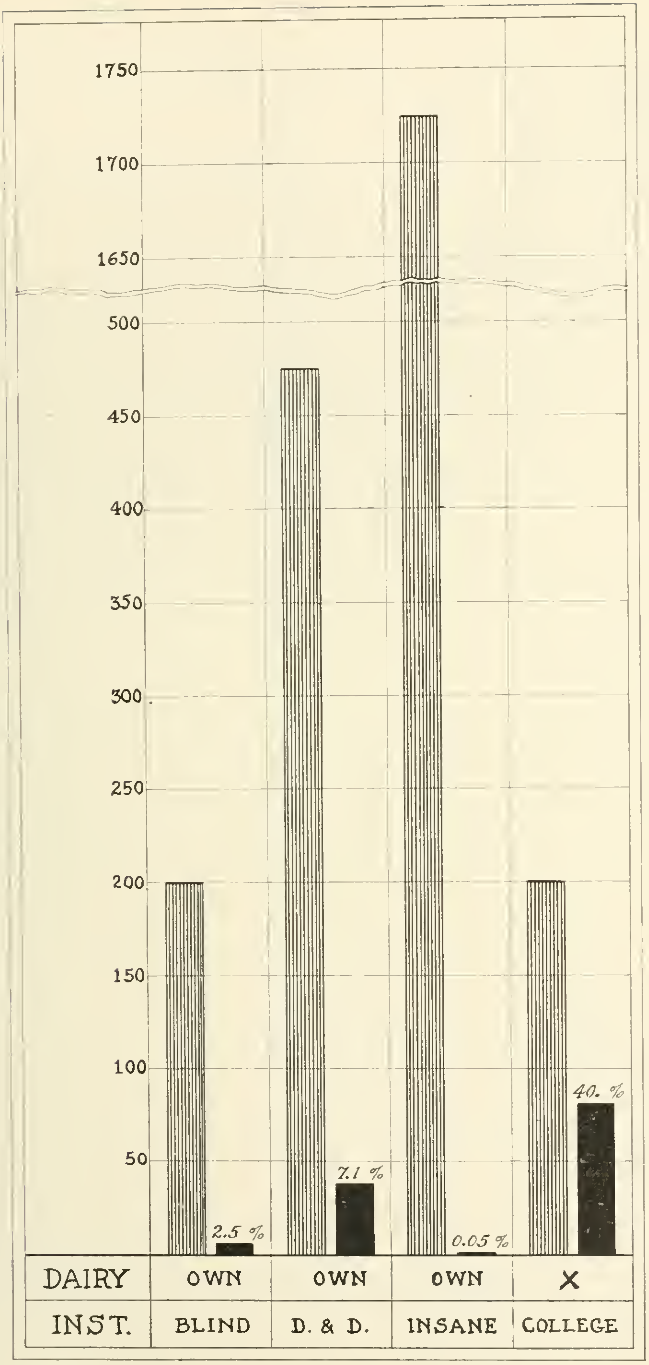

Chart 2.—Number of residents in several large institutions, the comparative prevalence of sore throat and the milk supply of each. Lined columns = number of residents. Black columns = number of cases of sore throat. Note the high morbidity in the college supplied by Dairy X.

regarding the occupants of a college dormitory and the permanent residents of a hospital. Both of the latter institutions depended on outside sources for milk.

Table 1 and Chart 2 bring out sharply the high morbidity in the college. Most of the students were attacked between November 29 and December 21. On one day eighteen were taken ill. In a total of 200 students, eighty, or 40 per cent., were victims.

The college was supplied with milk and cream by the aforementioned Dairy X.

The morbidity in the four other institutions varied from less than 1 per cent. to 7.1 per cent., averaging 1.7 per cent.

An interesting situation at the Insane Hospital should be noted. Twenty-five attendants and other employees, of a total of 220, consulted the house physician for sore throat. These persons often had occasion to eat and drink outside the hospital premises. In spite of close daily contact of these attendants with their charges, only one of the 1,725 patients contracted sore throat during December and January. The element of contagion from one person to another seems to have been negligible.

Again, it may be emphasized that the morbidity of sore throat in the college supplied by Dairy X was over twenty times that of the four other institutions supplied by other dairies.

When a summary is made of 7,416 individuals investigated both in households and institutions, we find that approximately 1,000, or 13.5 per cent., were customers of Dairy X. Of the total of 348 cases of sore throat 215, or 62 per cent., were users of Dairy X milk (Table 2).

TABLE 2.—Summary of Investigation

Total number of individuals investigated	7,416
Number of individuals using milk of Dairy X	1,000
Number of individuals using milk of other dairies	6,416
Total number of cases of sore throat	348
Cases of sore throat using milk of Dairy X	215
Cases of sore throat using milk of other dairies	133
Percentage of individuals supplied by Dairy X	13.5
Percentage of cases of sore throat using milk of Dairy X	62.0

Furthermore, among the 6,416 customers of other dairies, 133, or 2.1 per cent., developed sore throat, while among the 1,000 customers of Dairy X 215, or 21.5 per cent., contracted the disease (Chart 3).

2. INSPECTION OF COWS AND DAIRIES

For the inspection of the cows and dairies we were fortunate in securing the services of Mr. Kruger, an experienced dairy inspector in the Chicago health department.

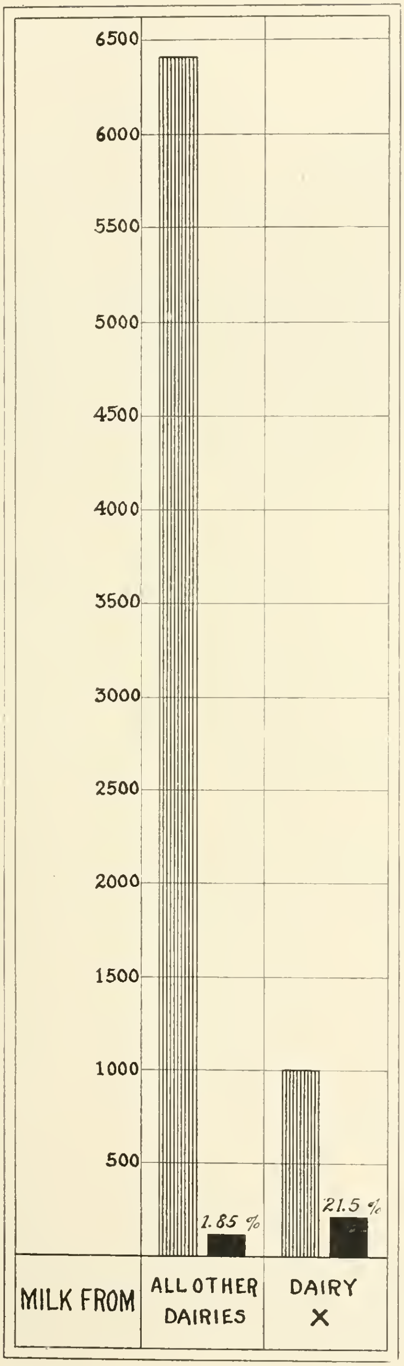

Chart 3.—Comparative morbidity of sore throat in the consumers of milk from Dairy X and from all other dairies.

In previous epidemics of septic sore throat the dairy investigation has been limited to those herds supplying milk that was under suspicion. Such a procedure fails to give information of the prevalence of garget in other herds as a basis of comparison. Furthermore, it is important to know if streptococcus mastitis may exist without being pathogenic to man. For these reasons the scope of dairy inspection was broadened so as to include all the cows in the larger dairies supplying the city.

On each farm it was ascertained if any members of the farmer's household had been afflicted with sore throat. The cows were examined carefully for caked bag or garget. Whenever garget was discovered, samples of the milk were taken separately in sterile tubes from each quarter of the bag under aseptic precautions, tightly sealed and forwarded at once to the Laboratory of Experimental Medicine, University of Illinois, Chicago.

In this manner inspection was made of 416 cows in thirty-eight different farms (Table 3).

Eleven cows were found with garget, which is about 2.6 per cent.

As the prevalence of garget in the Chicago epidemic was 4.6 per cent., we are not warranted in assuming that this disease was unusually common in the cows near Jacksonville. All of the cows with mastitis were placed under quarantine by the Milk Committee of the local medical society. Two of the eleven cases were caused by streptococci. These, it will be observed, came from farms that delivered milk to Dairy X.

In the farmers' families, six cases of sore throat were reported. None of them were milkers. All appeared after the outbreak of the epidemic. We think it is reasonable to assume, therefore, that these cases had no causal relation to the epidemic. Careful investigation, furthermore, failed to reveal any cases of sore throat about the dairies or distributing plants which could be brought into relation in any way with the outbreak of the epidemic. We thoroughly appreciate the importance of human "carriers" as possible sources for the infecting streptococci, but we could obtain no evidence that such persons played any rôle in the epidemic.

THE PLANT OF DAIRY X

Dairy X obtained its milk and cream from many farmers. The company sells milk and butter and a great deal of cream. It furnishes cream to the largest manufacturer and distributor of ice cream in the city. At the creamery plant is a pasteurizing apparatus which subjects the milk to a temperature of from 140 to 145 F. for a short and entirely inadequate period. *Cream was not pasteurized at all during the winter.*

TABLE 3.—Inspection of Cows and Dairies

Dairy	No. of Cows	Sore Throat on Farms	Infected Cows	Infecting Organism	Remarks
1	45	0	1	Staphylococcus albus	Cow hurt on wire fence.
2	9	0	1	Staphylococcus albus	Milk very yellow in color.
3	13	Girl—Dec. 15 2 wks. duration	1 late in Nov.	Hemolytic streptococcus	Milk supplied to Dairy X.
4	13	0	1	Hemolytic streptococcus	Milk supplied to Dairy X.
5	4	0	1	Staph. albus and aureus Strep. viridans	
6	9	0	0		
7	28	0	0		
8	22	0	0		
9	11	0	0		
10	11	Boy—Jan. 26	0		
11	5	0	0		
12	6	0	0		
13	12	0	0		
14	2	2 boys—Jan. 14	0	....	Use X Dairy milk
15	12	0	0		
16	6	0	0		
17	4	0	0		
18	14	0	1 (suspicious)	No organism found	
19	6	0	0		
20	5	0	0		
21	7	0	0		
22	6	0	0		
23	5	0	0		
24	7	0	0		
25	4	1 child—Jan. 6	0		
26	4	0	0		
27	9	0	0		
28	6	0	0		
29	13	0	0		
30	10	0	1—old case	Not examined	
31	7	0	0		
32	34	0	0		
33	15	1 case–Jan. 1-14 Dur. 10 days	0 0	....	Milk to Dairy X.
34	17	0	0		
35	16	1 case–Dec. 25 Dur. 10 days	0 0	....	Milk to Dairy X.
36	7	0	1–Nov. 24	Staphylococcus albus	Milk to Dairy X.
37	6	0	2–Jan. 1-14, Dec. 20-13	Staph. albus and aureus	
38	6	0	1	Staph. albus, fine bacillus	

None of the employees at the plant suffered from tonsillitis during the winter.

3. BACTERIOLOGICAL INVESTIGATION

The clinical symptoms, the general course of the disease, the character of the epidemic and its evident connection with the milk-supply would suggest at once that we have to do with a streptococcus infection, similar in character to the Chicago, Boston and Baltimore epidemics. Furthermore, the reports of throat examinations made by local physicians indicated the presence of numerous diplococci and streptococci in the smears obtained from the patients. Diphtheria was suspected often in these cases, but the cultures nearly always gave negative results for *Bacillus diphtheriae,* thereby showing that the infection was due to some other germ, probably streptococcus.

Owing to the fact that the epidemic was on the decline when the study began, not so many cultures from patients were obtained as we wished. In six typical cases, however, streptococci in large numbers were found as the predominating organism in smears and cultures of throat swabs. In one case an abscess occurred in the glands of the neck from which a pure growth of this organism resulted. The characteristics of these organisms isolated from throats will be considered later in connection with a discussion of the organisms obtained from the milk (Table 4).

As already stated, in an inspection of 416 cows from dairies about Jacksonville, eleven animals were found with diseased udders, and samples of milk were obtained from ten for bacteriologic examination. For this purpose the teats were cleansed and the first milk rejected. The milk was then collected directly in sterile test-tubes and immediately sent to the laboratory for examination. The results are presented in Table 3. Two cows were found having streptococcus mastitis. They were on different farms. The organisms were present in large numbers in the milk together with many leukocytes. The presence of the pus was immediately detected in the form of a slimy sediment in the centrifuge tube. A small amount of blood was present in one of the samples. In one cow two quarters were infected, in the other animal the four quarters were involved. Staphylococci were found in the milk from seven cows. In one was a fine, small, Gram-negative bacillus in large numbers and in another animal was obtained in smears and cultures an anhemolytic streptococcus similar in many respects to the *Streptococcus viridans,* but with no definite pathogenic properties for animals.

The properties of the streptococci isolated from the throats and from the diseased cows have been studied and the results are given in Table 4. Of the six strains isolated from the throats, four grow like the streptococcus isolated during the Chicago epidemic two years ago.[1] The other two do not show these properties as distinctly and closely resemble the ordinary hemolytic streptococcus. The two strains isolated from the udders of the cows show capsules, a narrow zone of hemolysis, and are pathogenic for rabbits in doses of one to two slant agar tubes, producing multiple arthritis in from three to four days. They clearly belong to the *Streptococcus pyogenes* group and correspond to the epidemic streptococcus found in the throats. In Table 4 are given the properties of these various streptococci and their reactions on different sugars. The organisms from the cows and from the throats agree in practically every detail. For comparison, Nos. 11 and 12 are typical streptococci isolated two years ago during the Chicago milk epidemic of sore throat. It will be seen that they do not differ essentially from the organisms isolated in the Jacksonville epidemic.

TABLE 4.—Properties of Epidemic Streptococci from Throats and from Cows

No.	Origin of Culture	Hemolysis	Capsule	Pathogen. for Rabbits	Dextrose	Saccharose	Lactose	Mannite	Maltose	Inulin	Raffinose
1	Human 1 (Jacksonville)	+	+	+	+	+	+	0	+	0	0
2	Human 2 (Jacksonville)	+	+	+	+	+	+	0	+	0	0
3	Human 3 (Jacksonville)	+	+	+	+	+	+	0	+	0	0
4	Human 4 (Jacksonville)	+	+	+	+	+	+	+	+	0	0
5	Human 5 (Jacksonville)	+	0	+	+	+	0	0	+	0	0
6	Human 6 (Jacksonville)	+	+	+	+	+	+	0	+	0	0
7	Human 7 (Jacksonville)	+	0	+	+	+	+	0	+	0	0
8	Bovine 1 (Jacksonville) Dairy 3...	+	+	+	+	+	+	0	+	0	0
9	Bovine 2 (Jacksonville) Dairy 4...	+	+	+	+	+	+	0	+	0	0
10	Bovine 3 (Jacksonville) Dairy 5...	0	0	0	+	0	+	0	+	0	0
11	Epidemic Strep., human, Chicago epidemic	+	+	+	+	+	+	0	+	0	0
12	Epidemic Strep. Bovine, Chicago epidemic	+	+	+	+	+	+	0	+	0	0

VIABILITY OF GERMS IN MILK PRODUCTS

During the study of this epidemic, questions were frequently asked as to the infectivity of sour milk, buttermilk, butter, cream, ice cream, etc. A somewhat detailed study of this problem is now being made, which will be reported in full later. Here only some general statements will be made. During the process of souring, hemolytic and epidemic cocci are gradually killed, as may readily be shown by making plates at regular intervals. These organisms are killed in three hours or less by the acidity of sour milk (forty-eight hours after curdling) and buttermilk. They die out in ordinary butter in the course of a few days. This is also due to the acidity, for in neutral butter they live for a long time. In ice cream streptococci remain alive for at least three weeks and without any appreciable diminution in number or virulence. Ice cream would seem to be the medium *par excellence* for the transmission and preservation of dangerous streptococci. Separator cream contains more organisms than the whole milk, and the skimmed milk contains less. The sediment contains large numbers. These facts are explained thus: The fat particles as they ascend carry with them mechanically a certain number of streptococci and other germs. On the other hand, many organisms are thrown down by centrifugation. In this way the number of organisms in the cream and the sediment is increased, while in the skimmed milk there is a decrease. Hemolytic and epidemic streptococci ordinarily do not multiply to any extent at 20 C. (68 F). At 27 C. (80.6 F.) there is little or no multiplication during the first six hours in milk (sterile or commercial), but at the end of twenty-four hours, in sterile litmus milk there is considerable multiplication. In ordinary commercial milk containing the common milk bacteria this multiplication is largely inhibited by the growth of these contaminating germs. Therefore, probably under few or no conditions met in the ordinary handling of milk can any appreciable multiplication of hemolytic or epidemic streptococci occur.

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[1] Davis: Bacteriologic Study of Streptococci in Milk in Relation to Epidemic Sore Throat, Jour. Am. Med. Assn., 1912, lviii, 1852.

Even under most favorable temperature conditions multiplication of these streptococci in sterile milk does not begin for from two to three hours. It is, therefore, impossible for any appreciable increase of the dangerous streptococci to occur during the short time while the temperature of milk is being raised and lowered in the process of pasteurization.

SUMMARY

1. The epidemic of septic sore throat began abruptly, was most active in the first four weeks and gradually subsided after about eight weeks.

2. There were 348 cases of sore throat, most of them complicated by enlargement of the cervical glands and many by a marked exudate. Cultures from a number of throats showed a hemolytic streptococcus.

3. In a canvass of 913 households, in 60 per cent. of the cases of sore throats, milk or cream from Dairy X was used, although this dairy supplied only 17.5 per cent. of the households.

4. The morbidity rate in one institution supplied by Dairy X was 40 per cent.; in four other institutions supplied by other dairies it averaged 1.7 per cent.

5. Among 1,000 customers of Dairy X, 215, or 21.5 per cent., developed sore throat, whereas among 6,416 customers of other dairies, 133, or 2.1 per cent., developed the disease.

6. As a result of a systematic inspection of 416 cows on thirty-eight dairy farms, eleven cows with garget were found.

7. Bacteriological examinations revealed hemolytic streptococci in the milk of two of these diseased cows. The milk of both of these cows was distributed by Dairy X.

8. As Dairy X did not pasteurize the cream at all and the milk very inadequately, we may conclude that the streptococci originating in the bovine mastitis were probably responsible for the epidemic.

9. A study of these streptococci under varying conditions in the milk shows these characteristics:

(*a*) They do not appreciably multiply at room or warm temperatures because of the inhibiting influence of other milk bacteria.

(*b*) They are soon killed by the acidity of sour milk or buttermilk.

(*c*) They die out in ordinary butter in a few days, probably because of the acids present.

(*d*) They live in ice cream for at least three weeks without appreciable loss of numbers or virulence. Ice cream, therefore, is a peculiarly favorable medium for the dissemination of the germs.

10. Because of the extensive distribution of ice cream, manufactured from the Dairy X cream, we suspect that ice cream played an important rôle as a carrier in this epidemic.

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SPECIFIC HYPERLEUKOCYTOSIS

*STUDIES IN TYPHOID IMMUNIZATION. IV**

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We have already briefly described the specific and extreme grade of hyperleukocytosis that follows the intravenous injection of living typhoid bacilli in rabbits that have been immunized against this microorganism.[1] In the present communication we wish to consider more fully the detail and mechanism of this phenomenon and its analog in other types of protein immunization.

The importance of the polymorphonuclear leukocytes in both natural and acquired immunity against bacteria has been well and advisedly emphasized by Metchnikoff and his pupils.[2] The observations on the importance of leukocytes in immunity have dealt, however, with the qualitative rather than the quantitative response of these cells to the infecting agent. Our work, we believe, points out the existence and importance of a quantitative leukocyte response in cases in which recovery from an acute infection occurs abruptly.

The injection of apparently any foreign protein substance in normal animals may give rise to a hyperleukocytosis preceded or not by a leukopenia. Thus, Sacconaghi[3] found that subcutaneous injections of horse-serum in rabbits produces leukopenia followed by hyperleukocytosis. Simonds and Baldauf[4] found that the intraperitoneal injection of heated cultures of *Bacillus coli* or *B. pyocyaneus* in the peritoneal cavity of rabbits produces a rapid leukopenia in from twenty minutes to four hours followed by a hyperleukocytosis in two or three days of as high as 22,000, when the normal count was 12,000.

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* From the Hearst Laboratory of Pathology and Bacteriology, University of California.

* We were assisted in this work by Mr. O. A. O. Sharpe, student in medicine.

[1] Gay, F. P., and Claypole, E. J.: Specific and Extreme Hyperleukocytosis following the Injection of Bacillus Typhosus in Immunized Rabbits, Jour. Am. Med. Assn., 1913, lx, 1950; A Further Note on Specific Hyperleukocystosis in Immunized Animals, Proc. Soc. Exper. Biol. and Med., 1913, xi, 47.

[2] Metchnikoff: L'immunité dans les maladies infectieuses, Paris, Masson & Cie, 1901.

[3] Sacconaghi: Leucocitosi; organi; leucopoietici; immunita, Morgagni, Milan, 1905, xlvii, 282.

[4] Simonds and Baldauf: The Relation of the Opsonic Index to the Leukopenia and the Leukocytosis Following Injections of Heated Bacterial Cultures, Jour. Infec. Dis., 1909, vi, 38.

Our first evidence of specific hyperleukocytosis was obtained, as is so often the case, in experiments designed for another purpose. Our method of testing the comparative value of different typhoid vaccines, the results of which are to be found in the following article,[5] has been to estimate the relative resistance of rabbits immunized by a given preparation against becoming "carriers." We are able to produce this carrier condition in rabbits with regularity on intravenous injection of one-half a standard blood-agar culture of a certain strain of *B. typhosus*.[6] Normal or insufficiently immunized animals become carriers, whereas the well-immunized animals give sterile cultures from the blood and gall-bladder within twenty-four hours. It was in the hope of tracing the fate of these myriads of injected bacteria in the circulation of the immune animals that we began making smears from the peripheral circulation soon after injection. To our surprise the organisms were not demonstrable in a smear made in ten or fifteen minutes after the injection; coincident, total and differential leukocyte-counts showed that the white blood-cells, particularly the polymorphonuclears, also diminished in a surprising manner, in many cases to one-sixth or one-eighth of their original number. Subsequent counts at rather irregular intervals soon showed that a leukocytic crisis occurs in from eighteen to twenty-four hours, and further counts showed that this crisis is preceded by another less marked one at about twelve hours. From the first, the grades of leukocytosis obtained in immunized animals have been controlled by parallel injections of the same amount of the same culture in normal rabbits, and in the latter animals there has been a surprising uniformity of counts. In the case of immunized animals it is far more difficult to be sure that the crest of the leukocytic wave has been attained, as the maximal count is of very short duration, as has been clearly determined in a few cases in which counts were made at half-four intervals. In other words, the rise of specific hyperleukocytosis is so abrupt that its height may well be missed unless the counts are frequent. The mean, therefore, of the immune counts as plotted in Figure 1 depresses rather than exaggerates the maxima individually obtained, whereas the normal counts better represent a true average (Fig. 1).

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[5] Gay, F. P., and Claypole, E. J.: An Experimental Study of Methods of Prophylactic Immunization Against Typhoid Fever. Studies in Typhoid Immunization V, The Archives Int. Med., p. 671 this issue.

[6] Gay, F. P., and Claypole, E. J.: The "Typhoid Carrier" State in Rabbits as a Method of Determining the Comparative Immunizing Value of Preparations of the Typhoid Bacillus. Studies in Typhoid Immunization, I, The Archives Int. Med., 1913, xii, 613.

When the difficulties of counts for twenty-four hours at two-hour intervals are considered, it is not surprising that we have only seven counts in the immune animal and four in the normal that are fairly complete. Figure 1 is based on an average from counts in twenty-five immune and eight normal rabbits; the counts that were incomplete through the twenty-four-hour period were so divided in time as to fill the various hour intervals rather evenly.

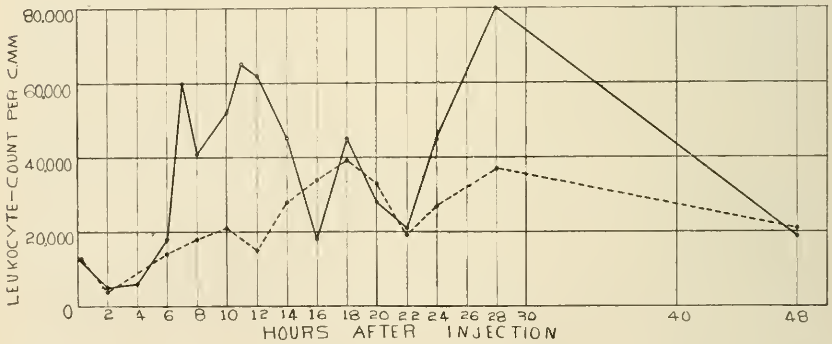
Some of the actual counts obtained are summarized in Table 1.

A differential count in either normal or immunized animals shows that both the leukopenia and hyperleukocytosis affects largely the polymorphonuclear type of cells. For example, in the counts of Immune Rabbit 108 given in Table 1, the differentials were as follows:

Before injection, total, 13,400: polymorphonuclears, 39 per cent.; small lymphocytes, 55 per cent.; large lymphocytes, 5 per cent.; eosinophils, 1 per cent.

Two hours after, total, 3,800: polymorphonuclears, 25 per cent.; small lymphocytes, 72 per cent.; large lymphocytes, 3 per cent.

Twenty-four hours after, total, 75,600: polymorphonuclears, 80 per cent.; small lymphocytes, 15 per cent.; large lymphocytes, 4 per cent.; eosinophils, 1 per cent.



Leukocytic response in normal and immune rabbits to injection of *Bacillus typhosus*. Immune rabbits are indicated by the solid line, normal rabbits by the broken line.

The abruptness of the leukocytic crisis in immune animals has already been mentioned; it may be illustrated by the following counts from Animal 65 on injecting the standard half blood-agar culture:

Leukocyte-count before injection, 13,800; two hours after injection, 6,400; eight hours after injection, 27,200; eleven hours after injection, 84,000; eleven and one-half hours after injection, 151,000; twelve hours after injection, 113,000.

That the exaggerated response in the typhoid-immune animals is specific is shown by an experiment in which a normal and an immune rabbit (typhoid) were given intravenous injections of one-tenth killed (60°) culture of *Micrococcus aureus*. The counts ran absolutely

TABLE 1.—Leukocyte-Counts in Normal and Immune Animals on Injecting One-Half Blood-Agar Culture Typhoid No. 3b

Rabbit	Before	Hours After Injection											
		2	4	6	8	10	12	14	16	18	22	28	48
Normal 1	13,000	4,400	3,800	31,800	50,400	45,000	22,000	22,800	39,000	49,000	15,400	30,000	15,200
Normal 2	14,000	4,200	11,000	16,400	28,500	19,000	15,000	21,500	18,400	50,000	18,800	14,000	14,200
Normal 126	12,000	.....	......	4,000	5,200	13,400	16,000	46,000	33,000	25,000	......	......	......
Normal 127	14,200	.....	......	6,400	5,700	6,600	15,000	20,000	34,000	33,000	......	......	......
Immune 202	19,600	5,000	6,000	14,900	27,000	27,600	63,000	40,800	22,000	145,000	72,000	48,000	16,800
Immune 203	14,200	4,600	6,200	4,400	8,800	24,000	16,600	30,000	27,000	57,000	42,800	30,200	20,000
Immune 65	13,800	6,400	10,000	14,200	27,200	57,400	151,000	......	......	....	......	21,600	13,200
Immune 108	13,400	3,800	......	33,600	85,600	......	....	......	......	....	......	25,000	10,400

parallel and showed little or no leukopenia and only a slight grade of leukocytosis in either animal.

A specific response in the typhoid-immune animals may be produced by the injection of various typhoid vaccines as well as by the living culture.

SPECIFIC HYPERLEUKOCYTOSIS IN OTHER TYPES OF IMMUNITY

Before considering more attentively the mechanism of this specific, quantitative reaction on the part of leukocytes, we may outline our experiments with the phenomenon in other types of immunity.

It can be shown that rabbits that have been immunized against red blood-cells of the sheep or guinea-pig react with a specific hyperleukocytosis to intravenous reinjection of the homologous cells. There is, of course, danger that animals so treated may die of anaphylactic shock, but intraperitoneal reinjections designed to obviate this danger did not give very striking results. Some of the more striking examples of red blood-cell leukocytosis are summarized in Table 2.

TABLE 2.—Leukocyte Response of Rabbits Immunized Against—

Rabbit No.	Immunized Against	Hemolytic Titer	Reinjection
131	Sheep blood	....	Sheep blood 1 c.c. intravenously
132	Sheep blood	....	Sheep blood 1 c.c. intravenously
196	Sheep blood	1 : 4,000*	Sheep blood 1 c.c. intravenously
170	Control	....	Sheep blood 1 c.c., intravenously
198	Control	....	Sheep blood 1 c.c., intravenously
1	Guinea-pig blood	1 : 2,000†	Guinea-pig blood 1 c.c., 5 pct...
4	Control	....	Guinea-pig blood 1 c.c., 5 pct...
2	Guinea-pig blood	1 : 2,000	Sheep blood 1 c.c., whole........

* Determined with 1 c.c. of a 5 per cent. suspension of sheep blood and 0.1 c.c. guinea-pig

† Determined with 1 c.c. of a 5 per cent. suspension of guinea-pig blood and 0.2 c.c. rabbit

It is evident from Table 2 that the phenomenon of specific hyperleukocytosis occurs with blood-cells as well as with bacteria, and the response would seem distinctly earlier. Our counts were designed simply to show that the phenomenon exists and are not so complete as in the case of the counts in the typhoid-immune rabbits; there is, in particular, no certainty that a second and higher crisis may not have been overlooked between ten and twenty-eight hours.

In view of Metchnikoff's findings of the phagocytic relations between macrophages (large mononuclear cells, endothelial cells and the like) and red blood-corpuscles, we had thought it possible that this type of leukocytosis might bear some relation to a relative increase in this type of leukocytes. As far as our differential counts have gone, however, the same relative and absolute increase in polymorphonuclear cells as noted in the typhoid animals has been found.

In the case of serum immunity, we failed at first to catch the period of hyperleukocytosis, or else it failed in the first animals because they had not been sufficiently highly immunized. It was later clearly determined in two well-immunized animals by means of counts at short intervals (Table 3).

MECHANISM OF SPECIFIC HYPERLEUKOCYTOSIS

The well-known tropic action of immune serum as an adjuvant to phagocytosis suggested early in our studies that we might here be dealing with a similar phenomenon, the tropins in the circulation of the immune animal rendering the injected bacteria more attractive to leukocytes and, therefore, increasing their response. If this hypothesis were true, we should expect to be able to evoke a similar response in normal animals on the injection of sensitized or tropinized bacteria or red blood-cells. Such has proved to be the case as is shown by the following experiments:

—Red Blood-Cells to Their Respective and Other Corpuscles

Leukocyte-Counts by Hours										
Before	1	2	3	4	5	7	8	9	10	28
18,400	.....	20,600	....	33,000	34,500	47,800	41,000	42,000	......	18 200
12,600	......	22,800	....	23,000	39,000	70,000	114,000	60,000	......	12,400
10,000	......	60 000	....	61,000	41,000	27,300	15,200	......	15,400	
5,660	......	10,400	....	9,600	8,800	9,200	6,500	......	7,000	8 000
12,000	......	24,000	....	26,000	25,000	14,000	....	......	15,000	
12,500	15,600	64,000	126,000	9,000	38,000	12,400				
9,200	9,600	16,400	32,000	11,000	29,000	20,000				
8,000	16,200	13,200	15,600	9 200	14,000	17,000				

alexin.
alexin.

Experiment 1.—A. To 1 c.c. of carefully washed sheep red blood-corpuscles was added 2 c.c. of a strong inactivated (56°) rabbit anti-sheep serum, and the mixture allowed to stand at 37 C. for one hour.

B. To the same amount of the same washed blood were added 2 c.c. of normal rabbit serum (56 C. or 104.8 F.) and the same contact allowed. The treated red blood-cells in both mixtures were then thoroughly washed three times in 0.85 per cent. sodium chlorid solution to remove serum. Two normal rabbits, Nos. 7 and 6, were given 1 c.c. intravenously of sensitized corpuscles

TABLE 3.—LEUKOCYTE RESPONSE IN SERUM RABBITS IMMUNIZED AGAINST HORSE-SERUM ON REINJECTION

Rabbit No.	Before	Leukocyte-Counts by Hours						
		1	2	3	3½	4	5	6
129........	15,400	23,200	92,200	106,800	35,000	12,800		
128........	10,200	6,100	10,600	....	28,400	30,000	70,000	40,000
Control....	13,900	4,600	4,300	....	5,700	6,600	5,900	......

TABLE 4.—LEUKOCYTE-COUNTS, RABBITS 7 AND 6

Rabbit No.	Injection	Leukocyte-Counts by Hours*					
		Before	1	2	3	4	5
7	Sensitized blood..	......	33,000	28,000	27,600	24,000	16,800
6	Unsensitized blood	14,000	70,000	194,000	28,000	12,000	......

* Titer about 1 : 4,000.

TABLE 5.—LEUKOCYTE-COUNTS BY HOURS, NORMAL RABBIT 10*

Before	1	2	3	4	5	6	7	8	9	10	11	12	13
10,400	9,000	5,400	9,000	4,200	6,800	9,600	6,600	11,200	12,600	26,400	20,800	130,000	19,000

* Agglutination titer, 1 : 4,000.

(*a*) and unsensitized corpuscles (*b*), respectively. Their leukocyte counts at following intervals are given in Table 4.

It is evident that the animal treated with sensitized blood showed a rapid rise of leukocytes to a maximum of 194,000 per cubic millimeter at two hours and then dropped rapidly to normal. The control treated with the same blood treated by a normal instead of an immune serum showed a nominal rise only. Similar, though not quite so striking, results have been obtained on injecting sensitized guinea-pig cells.

The phenomenon produced by injecting sensitized cells in normal animals may likewise be demonstrated on injecting sensitized bacteria.

Experiment 2.—A twenty-four-hour standard agar-culture of *B. typhosus* No. 5 was suspended in 2 c.c. of 0.85 per cent. sodium chlorid solution. To 1 c.c. of this suspension was added an excess of inactivated rabbit-antityphoid serum (1 c.c.). After contact for one hour at 37 C. (98.6 F.), the sensitized and clumped bacteria were washed free of serum by three washings in saline. (The effect of the same amount of untreated culture when injected intravenously in normal rabbits may be seen in Table 1).

The response of Normal Rabbit 10, given the sensitized culture, is given in Table 5.

This animal showed the characteristic high leukocyte-count of 130,000, twelve hours after injection, later than in the case of red blood-cells and corresponding to the rise obtained in immunized animals on injecting untreated bacteria.

THE POSSIBLE THERAPEUTIC SIGNIFICANCE OF AN ARTIFICIAL LEUKOCYTIC CRISIS

The work we have done with the specific leukocytic crisis, particularly the last fact, that it may be induced in normal animals by the use of sensitized (tropinized) cultures, immediately suggests therapeutic possibilities. Although our attention has hitherto been directed largely toward the prophylactic use of various typhoid vaccines, our preliminary experiments in animals in a curative way may be of suggestive value.

Metchnikoff has assumed that the principal locus of origin of the immune bodies is in the leukocytes. Our own experiments with determinations of the hemolytic and precipitin titer in blood- or serum-immunized animals, paralleling the leukocyte response on reinjecting the antigen (Tables 2 and 3), indicate that the crisis is followed by an increase in antibodies which rapidly restores the titer to as much or more than the original strength, which at first always suffers a temporary fall. In other words, the leukocytic crisis (and destruction?) would seem to liberate antibodies. We have already indicated that coincidently with the crisis in well-immunized typhoid animals the body sterilizes itself of the injected bacteria.

Similar processes are recognized as taking place in pneumonia, which is characterized by a crisis followed by rapid recovery. Dochez[7] has found that the protective value for mice of serums from human cases of pneumonia is increased after the crisis. Hektoen[8] also suggests the function of the leukocytic crisis in pneumonia as of curative value.

We have in a few instances been able to abort the usual carrier condition in rabbits that follows the injection of our standard dose of blood-agar culture of *B. typhosus* by injections of a sensitized typhoid vaccine, following the initial infection. Further study in this direction is at present engaging our attention.

Note in Conclusion.—In a recent article, Hamburger[9] has reported the leukocyte counts in children suffering from gonorrheal vulvovaginitis following intravenous injection of small amounts of a polyvalent gonococcus vaccine. A marked leukocytosis ranging from 15,000 to 28,000 was noted at the eighth hour, preceded by a hypoleukocytosis of from 6,000 to 7,000 at forty-five minutes. These results correspond to the results which we have obtained in immunized rabbits on reinjecting the antigen, although Hamburger has obtained only the first and less significant rise.

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[7] Dochez: The Presence of Protective Substances in Human Serum During Lobar Pneumonia, Jour. Exper. Med., 1912, xvi, 665.

[8] Hektoen: The Mechanism of Recovery in Pneumonia, Jour. Am. Med. Assn., 1914, lxii, 254.

[9] Hamburger: Zur Vakzinebehandlung der kindlichen Gonorrhoe, Deutsch. med. Wchnschr., 1914, xl, 759.

AN EXPERIMENTAL STUDY OF METHODS OF PROPHYLACTIC IMMUNIZATION AGAINST TYPHOID FEVER

*STUDIES IN TYPHOID IMMUNIZATION. V**

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INTRODUCTION

Beumer and Peiper[1] were undoubtedly the first fully to appreciate the possibility of an active immunization against infection with the typhoid bacillus. In 1887 they were able to prove that mice that have recovered from a non-fatal infection with living typhoid bacilli are frequently protected against subsequent, larger, and usually fatal doses of the same organism. In their most successful experiment they found that the best results were obtained by the gradual increase in dosage on successive inoculations, and they further suggest that it may be possible to immunize by means of sterilized cultures, which, as had already been shown, contain the toxic principle of the typhoid bacillus. They raise the question as to whether it might not be possible to immunize human beings by means of gradually increasing amounts of such killed cultures. In the following year Chantemesse and Widal,[2] following the work of Salmon and Smith[3] on hog cholera, and of Roux and Chamberlain[4] on malignant edema, found that they could protect mice against infection with living typhoid bacilli by means of sterilized cultures of the organism.

The practical application of these experimental results in animals to the prevention of typhoid fever in human beings did not come until eight years later, following the discovery of the lysins by Pfeiffer. It was A. E. Wright,[5] who, in a preliminary publication in 1896 fol-

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1. Beumer and Peiper: Bakteriologische Stüdien über die ätiologische Bedeutung der Typhusbazillen, Ztschr. f. Hyg., 1887, ii, 110.

2. Chantemesse and Widal: De l'immunité contre le virus de la fièvre typhoïde, conférée par des substances solubles, Ann. de l'Inst. Pasteur, 1888, ii, 54.

3. Salmon and Smith: On a New Method of Producing Immunity from Contagious Diseases, Proc. Biol. Soc. Washington, 1884-1886, iii, 29; Centralbl. f. Bakteriol., Orig., 1887, ii, 543.

4. Roux and Chamberlain: Immunité contre le septicémie conférée par des substances solubles, Ann. de l'Inst. Pasteur, 1887, i, 561.

5. Wright: On the Association of Serous Hemorrhages with Conditions of Defective Blood-Coagulability, Lancet, London, Sept. 19, 1906, ii, p. 802.

lowed by a fuller account by Wright and Semple[6] in the beginning of 1897, first outlined a method of immunizing human beings against typhoid fever. The method as outlined is notable on account not only of the essential facts involved, but also of the orderly and systematic method by which the problem was approached. Wright grew the culture of the typhoid bacillus in bouillon for two or three weeks and then killed them by heating to 63 C. (145.4 F.) for an hour, and preserved them with 0.5 per cent. phenol (carbolic acid). These vaccines were then tested for sterility and their toxicity carefully standardized by determining the minimal lethal dose for guinea-pigs; the dose chosen for injection in human beings was measured by this toxicity for animals. Wright further introduced a method of counting the number of the bacteria in the preparation employed by comparing their number in a given dilution when mixed with a suspension of red blood-cells, the number of which could be accurately determined. The dose of bacteria usually employed was from 750 to 1,000 million.

In the same year (1896) Pfeiffer and Kolle[7] described their method of immunizing and their method of estimating the protection that was produced in human beings by the agglutinins and the bactericidal potency of the serum. They employed agar-cultures of an avirulent strain of the typhoid bacillus suspended in salt solution and killed by heating to 56 C. (132.8 F.). An amount of this suspension, corresponding to one-tenth of an agar culture of something like 2 mg., was usually given on the initial injection, which was, as a rule, the only one. It is frankly admitted that the symptoms produced by this amount of culture were severe, and the method has since been modified in several ways to avoid these symptoms without essentially changing the principle involved.

So much for the first two communications on typhoid immunization in human beings. They form the groundwork on which subsequent methods of vaccination against typhoid fever have been built. The various methods that have since been advocated are numerous. Metchnikoff and Besredka[8] estimate that at least twenty different methods of vaccination have been described and advocated. Friedberger,[9] in his systematic review on typhoid immunization, enumerates twelve recognized methods. Paladino Blandini[10] has actually attempted to test the comparative immunizing value of seventeen preparations. It is not our purpose to describe all these methods in detail, and the reader who wishes further information on them may consult the systematic description of Friedberger[9] or of Fornet[11] in regard to them. It will be well, however, to outline the most important of these methods as evidence of the scope that the investigation has taken in perfecting this type of immunization, and as indicating the tendency which would seem to be leading to its gradual perfection.

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[6] Wright and Semple: Remarks on Vaccination Against Typhoid Fever, Brit. Med. Jour., Jan. 30, 1897, i. p. 256.

[7] Pfeiffer and Kolle: Experimentelle Untersuchungen zur Frage des Schutzimpfung des Mensches gegen Typhus abdominalis, Deutsch. med. Wchnschr., 1896, xxii, 735.

[8] Metchnikoff and Besredka: Recherches sur la fièvre typhoïde expérimentale, Ann. de l'Inst. Pasteur, 1911, xxv, 193.

[9] Friedberger: Die Methoden der Schutzimpfung gegen Typhus, etc. Kraus and Levaditi: Handbuch der Technik und Methodik der Immunitätsforschung, Fischer, 1908, i, 722.

PREPARATIONS OF THE TYPHOID BACILLUS THAT HAVE BEEN USED AS VACCINES

A. KILLED CULTURES OF THE TYPHOID BACILLUS

We have already mentioned that the first two preparations, those of Wright and of Pfeiffer and Kolle, consist essentially in killed cultures, the one, a bouillon-culture and the other a suspension of an agar-culture. These two original methods have been followed by many modifications. Thus Loeffler[12] took advantage of the fact that ferments, when dried, resist heating to a considerable degree without deterioration, and, regarding the antigenic property of the bacillus as ferment-like, dried suspended agar-cultures of the micro-organism and then heated them to from 120 to 150 C. (248 to 302 F.). These dried cultures were then pulverized and used in weighed amounts for immunizing animals. He states that such a culture has lost little of its property to produce antibodies. Friedberger and Moreschi[13] use a similar dried and heated culture, and administer it intravenously in very small doses; for example, an amount corresponding to 1/4,000 oese in immunizing human beings. It should be noted at this point that the method currently employed of determining the immunizing value of these preparations lies in an estimation of the antibodies (agglutinins, lysins, etc.) produced. As we shall later have cause to consider, these estimations offer an indication rather of the reaction of the animal body than a sure means of determining the degree of protection that has actually been afforded.

As we shall see in a moment, the use of living instead of dead cultures has been warmly advocated by certain observers, and their

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10. Blandini, Paladino: Profilassi specifica del tifo addominale, Ann. d'ig. sper., 1905, xv, 295.

11. Fornet: Immunität bei Typhus; Kolle and Wassermann: Handbuch der pathologische Mikroorganismen, Ed. 2, Fischer, 1912, iii, 837.

12. Loeffler: Ueber ein neues Verfahren zur Gewinnung von Antikörpern, Deutsch. med. Wchnschr., 1904, xxx, 1913.

13. Friedberger and Moreschi: Vergleichende Untersuchungen über die aktive Immunisierung von Kannichen gegen Cholera und Typhus, Centralbl. f. Bakteriol., Orig., 1905, xxxix, 453.

assertions have apparently convinced several who are not quite willing to adopt such preparations owing to their possible danger, although they endeavor to approach them as far as possible without actually using living micro-organisms. It is apparently now the consensus of opinion that bacterial cultures are most antigenic when employed as nearly as possible in their living, unaltered condition, and that heat in particular tends to alter or destroy essential, characteristic, antigenic properties. Several methods have been advocated for avoiding or obviating so far as possible the destructive influence of heat, and at the same time killing the bacteria. Leishmann[14] advocates killing the typhoid bacillus at 53 C. (127.4 F.) instead of at 56 or 60 C. (132.8 or 140 F.). Vincent[15] while fully recognizing the superior value of living cultures, regards their use as dangerous, and therefore kills the vaccines that he employs by means of ether. As will be later mentioned, we have used alcohol for the combined purposes of killing the typhoid bacilli and accelerating their flocculation and drying. Levy and Bruch[16] killed their preparations by shaking the micro-organisms in a medium containing galactose, and find that organisms prepared in this way immunize guinea-pigs, as well as the living cultures, and that these two preparations are far superior to killed cultures in corresponding amounts. Fornet[11] regards the unpleasant effects that are produced by the heated vaccine as due not only to the heating itself but also to the presence of a large amount of albumin in the culture-medium. He therefore grows his micro-organism in a medium containing only a small amount of peptone and kills them by heating to 55 C. (131 F.) for fifty-five minutes. Courmont and Rochaix[17] killed their preparations by heating to 53 C. (127.4 F.), and further modified the usual method by administering the antigen through the rectum. Nicolle, Conor and Conseil[18] heat their bacteria to 55 C. (131 F.) for forty-five minutes, and then to 52 C. (125.6 F.) for thirty minutes more, and inject intravenously. Wassermann[19] insists that the antibodies produced by typhoid bacilli heated to 53 C.

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[14] Leishmann: Preliminary Note on Anti-Typhoid Vaccine in the Treatment of Enteric Fever, Jour. Royal Army Med. Corps, 1909, xii, 136.

[15] Vincent: Sur la vaccination antityphique, Jour. State Med., 1912, xx, 322; Sur l'immunisation active de l'homme contre la fièvre typhoïde, Compt. rend. Acad. d. sc., 1912, clv, 480.

[16] Levy and Bruch: Vergleichende experimentelle Untersuchungen zwischen drei Typhusvaccinen, die so wohl Bakterienleibersubstanzen als auch lösliche Stoffwechsel produkte enthalten, Arbeit. a. d. k. Gsndhtsamte, 1913, xliv, 150.

[17] Courmont and Rochaix: Immunisation antityphique de l'homme par voie intestinale, Compt. rend. Acad. d sc., 1912, cliv, 611, 1829.

[18] Nicolle, Connor and Conseil: De l'inoculation intraveineuse des bacilles typhiques morts à l'homme, Compt. rend. Acad. d. sc., 1912, clv, 1036.

[19] Wassermann: Beiträge zur Typhus Schutzimpfung, Ztschr. f. Hyg., 1911, lxx, 204.

(127.4 F.) are not markedly better than when they are heated to 56 C. (132.8 F.). Renaud[20] has advocated the use of ultraviolet rays to kill the bacteria.

B. EXTRACTS OF BACTERIA

In addition to killed cultures of bacteria, numerous extracts and preparations derived from the bacteria have been advocated for the purpose of immunizing against typhoid fever. Hahn[21] has recommended the extract obtained from masses of bacteria by means of the Buchner press. McFadyen and Roland[22] have utilized liquid air as a means of killing bacteria and obtained from them an extractive substance. Neisser and Shiga[23] have utilized free receptors obtained by autolysis of bacteria at body temperature in salt solution. Wassermann[24] has suggested a similar method with the autolysis produced by distilled water. He subsequently dries the extract obtained in this way and uses it as a vaccine powder (*Impfpulver*). Brieger and Mayer[25] have used a watery, filtered extract of shaken bacteria. Bergell and Meyer[26] have used an extract of dried bacteria obtained by treating them with dilute hydrochloric acid.

Various so-called soluble toxins of the typhoid bacillus have also been suggested for immunizing purposes by Chantemesse,[27] by Werner,[28] and by Rodet, LaGriffoul and Wahby.[29] The extract of bacteria obtained by the method of Jez[30] has also been suggested.

C. LIVING CULTURES OF THE TYPHOID BACILLUS

Living cultures of the typhoid bacillus, usually more or less modified in their pathogenicity, have been warmly advocated by certain observers as producing the best immunizing preparations in a manner similar to the methods that have been employed in dealing with other diseases, notably in cholera (Strong and Kolle). Castellani[31] uses an avirulent strain of the typhoid bacillus in the form of recent bouillon-cultures which are then partially killed by heating to 50 C. (122 F.) for one hour. Such a modified culture produces rather severe local and general symptoms, but when given twice would, to judge from Castellani's results, produce a most satisfactory degree of immunity, which apparently has lasted in a number of cases on which he reports for at least four years. He suggests, as an alternative, that the first injection may consist of a killed culture followed by a living culture on the second inoculation. In addition to the superior immunizing properties of the living culture, it is also pointed out by Fornet[11] that the killed cultures in a given dose give more reaction because the split products of proteins, which are recognized to be toxic, are liberated by heat. Living cultures have also been employed by Pescarolo and Quadrone.[32] The form of living cultures which has been advocated by Besredka will be considered under the next heading. As has already been mentioned, living cultures are generally admitted to be of superior immunizing value by many who are not willing to adopt them, owing to the real or fancied dangers coincident with their use, and this has led to an attempt to approach the condition of living bacteria without actually employing them. (Compare Vincent,[15] Levy and Bruch,[16] and particularly Metchnikoff and Besredka, to be considered presently.)

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[20] Renaud: Vaccinothérapie par les vaccins irradiés (Etude biologique du vaccin typhique), Presse méd., 1911, xix, 655.

[21] Hahn: Immunisierungs und Heilversuche mit plasmatischen Zellsäften von Bakterien, München. med. Wchnschr., 1897, xliv, 1347.

[22] McFadyen and Roland: On the Intracellular Constituents of the Typhoid Bacillus, Centralbl. f. Bakteriol., Orig., 1903, xxxiv, 618.

[23] Neisser and Shiga: Ueber freie Receptoren von Typhus und Dysenterie Bazillen und über Dysenterietoxin, Deutsch. med. Wchnschr., 1903, xxix, 61.

[24] Wassermann: Zur aktiven Immunisierung des Menschens, Festschr. z. 60 Geburtst. v. Robert Koch, Fischer, Jena, 1904, p. 527.

[25] Brieger and Mayer: Weitere Versuche zur Darstellung spezifischen Substanzen aus Bakterien, Deutsch. med. Wchnschr., 1903, xxix, 309.

[26] Bergell and Meyer: Ueber eine neue Methode zur Herstellung von Bakterien Substanzen, welche zur Immunisierungszwecken geeignet sind, Med. Klin., 1906, xli, 753.

[27] Chantemesse: Toxine typhoïde soluble et sérum antitoxique de le fièvre typhoïde, Progrès méd., 1898, iv, 16.

[28] Werner: Sur la toxine excrété par le bacille typhique, Compt. rend. Soc. de biol., 1904, lvi, 836.

[29] Rodet, Le Griffoul and Wahby: La toxine soluble du bacille d'Eberth, Compt. rend. Soc. de biol., 1904, lvi, 794.

[30] Jez: Ueber Typhus Behandlung (Abdominal Typhus), mit einen antityphus Extract, Wien. med. Wchnschr., 1899, xii, 346.

D. SENSITIZED CULTURES OF THE TYPHOID BACILLUS

The method of active immunization by means of sensitized vaccines, that is to say, by cultures that have been first treated with an immune serum and then killed, was introduced by Besredka[33] in 1902. This method is not infrequently referred to as serovaccination, but it differs from the method properly called serovaccination suggested by Leclainche[34] in swine erysipelas and by Calmette and Salimbeni[35] in plague, in that the excess of immune serum which these authors used is removed from the treated bacteria. It was found, as Besredka notes, that this excess serum tends to produce simply a passive immunity instead of the active immunity which is produced by the cultures treated with immune serum, and washed. Apart from his original experimental work, Besredka did not deal with the practical aspects of sensitized vaccines until the experimental work on typhoid fever in apes was taken up by him in collaboration with Metchnikoff in 1911.[8] In the meantime, however, sensitized vaccines had been tried out apparently with considerable success in at least three instances. Marie[36] had been able to utilize the principle in treating rabies virus, Dopter[37] in vaccination against dysentery, and Theobald Smith[38] in a similar way found that he could produce active immunity by a balanced mixture of diphtheria toxin and antitoxin. This method of active immunization against diphtheria by a balanced (sensitized) mixture of toxin and antitoxin has recently been applied to human beings by von Behring.[39] The principal advantages of this method, as originally pointed out by Besredka and as apparently proved, are first, it produces little or no violent reaction on inoculation in instances in which the untreated bacteria themselves are distinctly irritating, as, for example, in plague. Secondly, it gives rise to an immediate though transitory passive immunity. Thirdly, it produces, eventually, an active immunity which is as enduring and as rapidly formed as when untreated bacteria are used.

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[31] Castellani: Typhoid and Paratyphoid Vaccination with Live Attenuated Vaccines, Lancet, London, 1912, i, 583; Observation on Typhoid Vaccination in Man with Attenuated Living Cultures, Centralbl. f. Bakteriol., 1909, lii, 92.

[32] Pescarolo and Quadrone: Aktive Immunisation durch subcutane Injektion lebender Typhusbazillen bei Eberthschen Infection, Centralbl. f. inn. Med., 1908, xxix, 989.

Not a little experimental work was done with sensitized typhoid vaccine before the work of Metchnikoff and Besredka, which will be taken up later. Paladino Blandini[10] made a very careful study of seventeen different typhoid vaccines, comparing their relative immunizing properties. His experiments were carried out on guinea-pigs, which were treated by the various preparations and subsequently given an intraperitoneal dose of living typhoid bacilli. The vaccines tested include small doses of living cultures; killed cultures prepared after the method of Pfeiffer and Kolle, and of Wright; several "soluble toxin preparations" of the typhoid bacillus; nucleo-albumins; and extracts of the typhoid bacillus prepared in different manners. Compared with these methods was the sensitized vaccine employed by

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33. Besredka: De l'immunisation active contre la peste, le choléra et l'infection typhique, Ann. de l'Inst. Pasteur, 1902, xvi, 918.

34. Leclainche: Sur la sérotherapie du rouget du porc, Compt. rend. Soc. de biol., 1897, xlix, 428.

35. Calmette and Salimbeni: La peste bubonique: Etude de l'épidémie d'Oporto en 1899, Ann. de l'Inst. Pasteur, 1899, xiii, 865.

36. Marie: Immunisation par des mélanges de virus rabique et de sérum antirabique, Compt. rend. Soc. de biol., 1902, liv, 1364.

37. Dopter: Vaccination préventive contre le dysentérie bacillaire, Ann. de l'Inst. Pasteur, 1909, xxiii, 677.

38. Smith, Theobald: Active Immunity Produced by So-Called Balanced or Neutral Mixtures of Diphtheria Toxin and Antitoxin, Jour. Exper. Med., 1909, xi, 241.

39. Von Behring: Ueber ein neues Diphtherie Schutzmittel, Deutsch. med. Wchnschr., 1913, xxxix, 873.

Besredka, and Blandini was able to demonstrate that the latter method was by all means the most protective. Not only were guinea-pigs protected for at least four months, but it was found that their serum, which contains sensitizers, also protects normal animals against infection. Ardin-Del-teil, Negre, and Raynaud[40] find that the use of sensitized typhoid vaccine in rabbits and human beings gives rise to relatively small amounts of agglutinins, but the bactericidal properties of the serum of those treated in this manner are much higher than of those treated by the ordinary cultures. These authors have also obtained very favorable results in treating cases of typhoid fever with this vaccine.

This failure of sensitized or agglutinated cultures to produce potent antibodies had already been noted by Neisser and Lubowski[41] and by Pfeiffer and Bessau.[42] Garbat and Meyer[43] immunized rabbits either with sensitized or with whole cultures and compared the properties of the serums of the two sets. The serum obtained by immunizing the rabbits with sensitized cultures agglutinated and gave the fixation reaction much less strongly than the corresponding animals treated with unsensitized cultures. The serums of the sensitized animals, however, were more bacteriotropic and protected animals experimentally much better than the serum of the animals treated with the plain vaccine. This fact is incidentally evidence of the unreliability of the agglutinin test as a measure of the grade of protection against typhoid infection, a matter which we shall later consider.

In 1911 Metchnikoff and Besredka[8] first reported their very important work on experimental typhoid fever in anthropoid apes. They found that the chimpanzee and the gibbon when given the dejecta from cases of typhoid fever, or lavishly contaminated food, or, in their latter experiments, when the mouth of the animal is painted with cultures of the typhoid bacillus, after eight days' incubation develop characteristic typhoid fever. Fifteen or sixteen monkeys that were tested gave positive blood-cultures on the tenth day, their serum contained agglutinins, and, in three instances, death due to the typhoid infection followed. The temperature of these animals ran as high as 40.8 C. (105.4 F.). Peyer's patches were found swollen but not ulcerated. The only detail in which this experimental disease would seem to differ from typhoid fever in human beings is the fact that the spleen in the chimpanzees is not distinctly enlarged. Having determined in this way that the typhoid bacillus is in reality the cause of typhoid fever and not some adherent filterable virus, as in the case of hog cholera, they turned their attention to methods of immunization against the disease. Their first series of experiments are far from convincing, although they draw from them rather sweeping conclusions. In the first place, the number of animals employed was necessarily small owing to their expense and the great susceptibility of the animals to extraneous infection, so that in this first series, in which there were five experiments, there was only one vaccinated animal in each case, tested with an untreated control. They come to the conclusion from their preliminary attempts that neither Vincent's typhoid vaccine, an autolysate of the typhoid bacillus, nor a *killed* culture sensitized according to the method of Besredka, will immunize anthropoid apes against a subsequent infection by the mouth.

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[40] Ardin-Del-teil, Negre and Raynaud: Sur la vaccinotherapie de la fièvre typhoïde, Compt. rend. Acad. d. sc., 1912, clv, 1179; Recherches sur les réactions humorales des maladies atteints par la fièvre typhoïde traités par le vaccin de Besredka, Compt. rend. Soc. de biol., 1913, lxxiv, 371; Récherches cliniques et expérimentales sur la vaccinothérapie de la fièvre typhoïde par le virus sensibilisé de Besredka, Ann. de l'Inst. Pasteur, 1913, xxvii, 644.

[41] Neisser and Lubowski: Lässt sich durch Einspritzung von agglutinierten Typhusbazillen eine Agglutinproduktion hervorrufen? Centralbl. f. Bakteriol., Orig., 1901, xxx, 483.

[42] Pfeiffer and Bessau: Zur Frage der Antiendotoxine bei Thyphus abdominalis, Centralbl. f. Bakteriol., Orig., 1910, lvi, 344.

[43] Garbat and Meyer: Ueber Typhus Heilserum, Ztschr. f. exper. Path. u. Therap., 1910, viii, 1.

Apart from the necessarily small number of animals in each experiment, certain other objections may be made to their experiments and conclusions, part of which Vincent[44] has pointed out. In the first place, the animals were tested very shortly after finishing the immunizing treatment, usually in from four to six days, which may reasonably be regarded as too brief a period for the establishment of the highest grade of immunity; and, secondly, the dose given the animals by the mouth, which is not stated very definitely, seems in all events to be extremely large, much larger, indeed, than in the natural infection in man. They conclude from their experiments that heated vaccines do not protect anthropoid apes against typhoid infection, and, owing to the analogy of the disease in these animals with the human syndrome, they regard such vaccines as unfitted to protect human beings. Inasmuch as the protective vaccination of man against typhoid fever by means of heated vaccines is generally recognized as reducing the morbidity to one-half or one-sixth of the normal, the logic of their position does not seem sound. It would seem better to have concluded that the immunization of the disease in anthropoid apes, as they have produced it, is not similar to their analogs in human beings.

In their second communication, Metchnikoff and Besredka[45] present experiments which, with the exception of one experiment in which Vincent's vaccine is used, are more convincing. Their second series deals with the protective effect of vaccination by a living paratyphoid B culture, and by sensitized *living* cultures of the typhoid bacillus against subsequent infection of the latter organism in the manner described. These experiments comprise more animals than the previous series, and in addition the infection is not given until from ten to fifteen days after the completion of the treatment. Vincent's culture again failed to protect, whereas the living sensitized typhoid culture and the living paratyphoid B cultures uniformly saved the animals. It would be far from safe, however, to conclude from these experiments that no preparation of heated typhoid bacilli has the power to protect even anthropoid apes against subsequent infection by the typhoid bacillus.

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[44] Vincent: Remarques sur la vaccination antityphique, Ann. de l'Inst. Pasteur, 1911, xxv, 455.

[45] Metchnikoff and Besredka: Des vaccinations antityphiques, Ann. de de l'Inst. Pasteur, 1911, xxv, 865.

We have examined the experimental details of the work of Metchnikoff and Besredka not in a spirit of unfavorable criticism, because, as will later be shown, we personally believe that their conclusions were in part correct, though the data on which they have based them have not convinced us.

We may here examine the results that have already been obtained in immunizing human being by the living sensitized cultures recommended by Metchnikoff and Besredka. Broughton Alcock,[46] working under their direction, was the first to report on the harmlessness of the method, its freedom from untoward effects and its apparent protective value. He advocates a dose corresponding to 1/100 of an agar-culture, which is equivalent to 500 million typhoid bacilli. Their organisms are sensitized by a few drops of a strong anti-typhoid serum, the exact potency and amount of which is not stated either by Metchnikoff and Besredka or by Broughton Alcock. After standing for twenty-four hours the clumped bacteria are washed in salt solution and resuspended in an aliquot portion of normal saline. A living sensitized vaccine prepared in this manner keeps for at least four months on ice. Objections have been raised by Vincent and others as to the danger of injecting living micro-organisms into the human body, but Metchnikoff and Besredka[47] have reported that careful tests fail to show that human beings treated in this manner become carriers of the typhoid bacillus or eliminate the micro-organism through the feces or urine. In view of the uniformly good results that have been obtained, there seems little reason further to suspect the harmfulness of the method. In a recent report Metchnikoff and

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46. Alcock, Broughton: Vaccination for Typhoid by Living Sensibilized Typhoid Bacilli, Lancet, London, 1911, ii, 497.

47. Metchnikoff and Besredka: Sur la vaccination contre la fièvre typhoïde, Compt. rend. Acad. d. sc., 1912, clv, 112.

Besredka[48] have further endeavored to determine the optimal vaccinating dose and interval of this sensitized vaccine, and Besredka[49] reports some of the favorable results that have been obtained. Cadeau[50] vaccinated twenty-five patients, and only two showed slight general symptoms. In an asylum at Briqueville where many cases of typhoid fever existed in 1912, 516 persons were vaccinated, and in the twelve months subsequent, no cases occurred among them whereas four occurred in the 343 persons who were not immunized. Marie reports similar absence of general reaction. In all, Besredka has dispensed 10,000 doses of the vaccine and no unpleasant results have been reported except when the injections were given intramuscularly. He recommends a dosage of from 500 million to 1,000 million, although larger amounts could be given without harm. It is, of course, too early to draw any conclusive figures as to the actual protective value of this method, although its harmlessness and freedom from untoward symptoms seem to have been proved.

PERSONAL INVESTIGATIONS

In view of the general acceptance of the relative protective value of immunization with killed cultures of the typhoid bacillus, and the claim that this method of vaccination with sensitized living typhoid bacilli insures all the advantages of the more unpleasant method of treating with dead cultures, and protects as well or better, it would seem desirable to devise some method of animal experimentation which would enable us to settle conclusively this and many other debatable questions that arise in connection with this extremely beneficial process. There are still many questions in connection with typhoid vaccination which remain unsettled, such as the dosage to be employed, the interval at which the injection should be given, and most important of all, the duration of the protection that is afforded by any particular method and to any particular individual. Animal experimentation has, to be sure, been used for the most part on guinea-pigs that after treatment with various typhoid vaccines are infected in the peritoneal cavity. It seems justifiable, however, to question the transference of results obtained in preventing a banal peritonitis in this animal to human beings. The method of testing typhoid vaccines on anthropoid apes that Metchnikoff and Besredka have described is undoubtedly the ideal one, but practically impossible to employ for the settling of details of method owing to the expense of the animals and the difficulty in obtaining them in any numbers.

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48. Metchnikoff and Besredka: Des vaccinations antityphiques, Ann. de l'Inst. Pasteur, 1913, xxvii, 597.

49. Besredka: Deux ans de vaccination antityphiques avec du virus sensibilisé vivant, Ann. de l'Inst. Pasteur, 1913, xxvii, 607.

50. Cadeau: Cited by Besredka (49), Ann. de l'Inst. Pasteur, 1913, xxvii, 607.

We have already described in some detail our method for testing the immunizing power of a given typhoid vaccine by means of the "rabbit-carrier" condition.[51] By employing one-half of a standard 10 per cent. blood-agar culture of a certain strain of *B. typhosus,* we have been able to produce this condition in normal rabbits with relative certainty. Thus of thirty control animals, twenty-eight, or 93 per cent., either became permanent carriers with positive cultures from blood or gall-bladder or, in a few instances, died acutely within twenty-four hours with symptoms of intoxication. For further discussion of the experimental advantages of this method over others proposed for this purpose, excepting experiments on anthropoid apes, we refer readers to our previous communication.

As we have just suggested, the presence of antibodies, particularly of agglutinins, is not a measure of the degree of protection against typhoid fever either in animals or in human beings. Antibodies indicate reaction which usually parallels protection but is by no means synonymous with it. In typhoid fever in particular the eventual absence of agglutinins in those recovered from and thereby protected from the disease indicates that serum tests are by no means to be confused with protection, which is more likely to be of a cellular than of a serum type. This indicates emphatically the necessity of testing the prophylactic immunizing value of any given vaccine by actual infection of the vaccinated animal rather than by any test of its antibodies. In our previous paper we have put forward our reasons for regarding the artificially produced bacteriemia in rabbits as analogous to typhoid fever in human beings, which with increasing agreement is now regarded primarily as a bacteriemia rather than a purely intestinal disease. The fact that we are able to prevent this carrier condition by previous immunization with one typhoid vaccine, whereas another preparation of the typhoid bacillus in the same amount does not prevent it, is further evidence that the method we have chosen is a delicate one and further suggests the variations in result that have been obtained in using different typhoid vaccines in human beings.

PREPARATION OF TYPHOID VACCINES FOR COMPARATIVE TESTS

The first essential in comparing various vaccine preparations seemed to us to lie in a standardization of the amount of substance employed. The methods usually employed in standardizing vaccines for clinical use may be accurate enough in view of the fact that the optimal dose is largely empirical and a little less or more may make no difference in the results. The fact that the usual methods of enumeration of bacteria by counting by the Wright method are being constantly changed is evidence enough that it is a far from satisfactory condition. We had already adopted the far more accurate method of dried, weighed bacterial preparations when the work of Wilson and Dickson[52] came to our notice. These authors have carefully determined the actual weights of the doses of various bacteria as commonly employed, checking their weighing results by counting the bacteria in a given volume of suspension. They find, for example, that there are on an average 8,000 million of typhoid bacilli in 1 mg. of a typhoid vaccine dried on platinum-foil. We were surprised to find how closely our estimations, although obtained in a different manner, agree not only among themselves, but also with the results of these authors. Our method of preparing dead typhoid vaccine has been to add normal saline (0.85 per cent.) in proportionate amounts to suspend forty-eight-hour cultures grown on Blake bottles containing 125 c.c. of 2 per cent. agar (titrated + 1) or, in certain experiments, of the same medium containing 10 per cent. fresh defibrinated rabbit blood. To such a suspension is added an equal volume of absolute alcohol, which kills the culture in the proportions used in less than fifteen minutes, flocculates out the organisms so that they can be easily collected by centrifugalization, and accelerates their drying in an unglazed porcelain plate in partial vacuum over sulphuric acid. Estimations of the number of typhoid bacilli in two different suspensions by counting (Wright's method) or by plating in successive dilutions, and by weight are summarized in the following experiments:

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[51] Gay, F. P. and Claypole, E. J.: The "Typhoid Carrier" State in Rabbits as a Method of Determining the Comparative Immunizing Value of Preparations of the Typhoid Bacillus, Studies in Typhoid Immunization I, The Archives Int. Med., 1913, xii, 613.

Experiment 1.—Vaccine 8: Nine Blake bottles containing 10 per cent. rabbit blood-agar were inoculated with a blood bouillon culture of *B. typhosus* 3b and incubated for forty-eight hours. The combined growths were suspended in a total of 220 c.c. of normal saline and shaken; 20 c.c. were kept for plating; 200 c.c. were precipitated with 200 c.c. of absolute alcohol, centrifugalized and dried *in vacuo* over sulphuric acid. The dried residue was ground for one hour in a mechanical ore-grinder (in a wooden case) sterilized by formaldehyd fumes and containing an agate mortar and pestle run by a small motor. This residue of microscopically disintegrated bacterial bodies weighed 675 mg.

By successive plates the bacteria in 1 c.c. of the original suspension were estimated to be 25,500 millions. In 200 c.c. the bacteria, weighing 675 mg., should equal 5,100,000 millions, or 7,555 millions to the milligram.

Experiment 2.—Vaccine 9: Nine Blake bottles of plain agar were inoculated with a bouillon culture of *B. typhosus* 3 and incubated for forty-eight hours. This growth was suspended in 200 c.c. of saline and the subsequent treatment was the same as in Experiment 1, except that the numerical estimation of the living bacteria was made by comparison with red blood-cells in a stained preparation (Wright's method).

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[52] Wilson and Dickson: Rapid Gravimetric Method of Standardizing Vaccines, Jour. Hyg., 1912, xii, 49.

The weight of dried ground culture equaled 360 mg. We draw attention again[51] to the fact that the growth on blood-medium of *B. typhosus* is approximately twice that of the growth on agar. By count a cubic centimeter contained 13,000 millions, or 2,600,000 millions to 200 c.c., giving 7,222 millions to the cubic centimeter.

These two estimates of 7,555 millions and 7,222 millions of typhoid bacilli to the milligram may be compared with the 8,000 millions of Wilson and Dickson. Our interest in these figures, however, lies not in proving that the various methods of counting bacteria in our own and others' hands give surprisingly concordant results, but as giving us a comparison of weight to number so that one may state roughly in experimental and clinical work the weight dosage of the typhoid preparation required to give an amount comparable to, say, the classical 500 million bacteria ordinarily employed. There can be no question that in experimental work weighed amounts of dried preparations are more comparable and easier to determine than counted suspensions. Assuming the counts of Wilson and Dickson to be more accurate, the weighed amount of dried typhoid bacilli corresponding to a dosage of 500 million bacteria would be $\frac{1}{16}$ mg.

In all our experiments with vaccine preparations, then, we have used weighed amounts of dried and ground bacilli as a basis for comparison, except in those cases in which living cultures of the organisms have been employed, in which case comparisons were made in another way.

METHOD OF IMMUNIZATION

Having determined on the use of weighed amounts of dried, ground bacteria for comparative testing of typhoid vaccines, the next essential was to decide on the best method of immunization of the rabbits that are to be tested for protection against the carrier state.

Fornet and Müller[53] and Bonhoff and Tsuzuki[54] have already shown that for the production of certain antibodies an intensive method of immunization may be employed by reinjection at short intervals. One of us (Gay), in collaboration with Fitzgerald,[55] has still further perfected this method. We were able, for example, to produce high-grade hemolysins to sheep corpuscles by injecting rabbits intravenously on three successive days with 1 c.c. of washed sheep blood. These lysins we find to be present in high degree four or five days after the last injection. Similar, although not quite so perfect, results were obtained in the production of precipitins and also in the production of bacterial agglutinins by Miss Edna Locke.[56] On the basis of these experiments, we had already determined that, provided a vaccine did not produce too severe symptoms, the period over which immunization extended might be markedly decreased without any corresponding deduction in the intensity of the immunity effected. It is of interest to note that Fornet[11] has also adopted a rapid method of immunization in vaccinating against typhoid. We have ourselves recommended, and Force[57] has employed immunization at intervals of two or three days in human beings, as will be mentioned later. The rapid method of immunization at short intervals not only may be shown in the case of rabbits to effect an equally high grade of immunity as determined either by antibody content or by actual resistance to infection, but also has the further advantage of actually producing less harmful effects when large amounts of the untreated vaccine are used. We have repeatedly noted that if a large total amount of vaccine is given to rabbits during a period extending over three weeks there is a larger percentage of mortality due to cachexia than when the same amount of vaccine is given within three days. The immunizing doses have invariably been three in number and have been administered either intravenously or subcutaneously; in all our later experiments it has seemed better to adopt the intravenous method as giving greater uniformity of results.

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[53] 53. Fornet and Müller: Zur Herstellung und Verwendung praezipitierenden Sera, inbesondere für den Nachweis von Pferdefleisch, Ztschr. f. biol. Tech. u. Methodik, 1908, i, 201.

[54] 54. Bonhoff and Tsuzuki: Ueber die Schnellimmunisierungsmethode von Fornet und Müller, Ztschr. f. Immunitätsforschung, 1910, iv, 180.

[55] 55. Gay and Fitzgerald: An Improved Rapid Method of Producing Precipitins and Hemolysins, Univ. Cal. Pub. Path., 1912, ii, 77.

The advantage of immunization at short intervals as against longer intervals was first tested by comparing the resistance to the carrier state of two sets of rabbits, the one immunized by three intravenous injections on three successive days, the other by three equal doses administered intravenously at three-day intervals. There was found to be no marked difference in relative resistance of these two sets of animals when given the test dose two months later. The agglutinins averaged the same titer in the two sets. In a more systematic experiment, a comparison of three daily with three five-day interval doses was made (Experiment 3).

Experiment 3.—Fifteen rabbits weighing from 2,000 to 2,500 gm. each were divided into two lots of eight and seven animals and immunized as follows:

Lot 1: Eight animals were given three doses of 1/6 mg. of dried, sensitized[58] Vaccine 13 intravenously in a volume of 1 c.c. 0.5. per cent. carbolated saline solution, Oct. 6, 13 and 22, 1913, that is, a total of 0.5 mg. in a period of sixteen days.

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[56] Locke, Edna: A Rapid Method of Producing Bacterial Agglutinins, Univ. Cal. Pub. Path., 1912, ii, 91.

[57] Force: Institutional Vaccination Against Typhoid Fever, Am. Jour. Pub. Health, 1913, iii, 750.

[58] Gay, F. P., and Claypole, E. J.: The Typhoid "Carrier-State" in Rabbits

Lot 2: Seven animals were given three doses of 1/6 mg. of dried, sensitized Vaccine 13 intravenously, Oct. 20, 21 and 22, in a volume of 1 c.c. 0.5 per cent. carbolated saline solution, that is, a total of 0.5 mg. in a period of three days.

Before test inoculation, in Lot 1, three animals died with extreme emaciation and no distinctive lesions (cachexia) — one of an intercurrent infection and one of injury. That is, in four of the eight animals death may reasonably be attributed to the vaccine. No animals in Lot 2 died during this period.

On the forty-fourth day after the end of immunization, the survivors of Lot 1, and those of Lot 2 were given each one-half standard blood-agar culture of *B. typhosus* 3b intravenously. Necropsies were performed on the animals that died, cultures were made from the blood and bile. The survivors were chloroformed, and cultures made from blood and bile on the ninth day. When organisms of proper morphology and cultural characteristics were isolated from either source they were tested by agglutination with a strong antiblood typhoid serum.[58] The results of this experiment may be summarized in Table 1.

TABLE 1.—RESULTS OF LONG AND SHORT-INTERVAL METHODS OF TYPHOID IMMUNIZATION IN RABBITS

	Died During Vaccination	Carriers	Recovered
Short intervals	0	3	4
Long intervals	4	3	0

This experiment as well as other similar ones would seem to indicate the superior value of the short-interval intensive method of immunization.

DOSAGE OF VACCINE PREPARATIONS

We have no contribution to make on the optimal dose of typhoid vaccine for prophylactic use in human beings. As will be seen later, our choice for such purposes has been largely empirical and has followed accepted usage. In our comparative experimental work, however, it has been necessary not only to use preparations derived from weighed, dried bacteria, but also to determine with increasing accuracy the duration of the protection afforded by a given amount of a standard preparation. In our first experiments we immunized separate series of rabbits each with a given preparation and then tested representatives of each series at several successive periods. Inasmuch as we started with rather large doses of vaccine, the time before the protection wore off was rather long and the method time-consuming.

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as a Method of Determining the Comparative Immunizing Value of Preparations of the Typhoid Bacillus. Studies in Typhoid Immunization I. Agglutinability of Blood and Agar Strains of the Typhoid Bacillus, Studies in Typhoid Immunization II, THE ARCHIVES INT. MED., 1913, xii, 621. See later, Experiment 4.

By decreasing the total vaccinating dose we were finally able to estimate with certainty that a given amount of one type of vaccine would protect for five or six weeks; with this point determined, it was easy to compare the immunizing value of other preparations with this one. In nearly all experiments, two or more control normal animals have been injected, although their use is not absolutely imperative since the less well protected animals become carriers and the percentage of carriers in each series gives the basis of comparison.

DETERMINATION OF THE TYPHOID VACCINE OF ELECTION FOR IMMUNIZATION

The carrier condition was at first determined in the immunized and control animals by cultures taken from the blood ten and twenty days after inoculation; the results were checked by the post-mortem condition and cultures from the bile when the animals died, or in the case of survivors by chloroforming them from fifty to seventy days later. A later method which gives quicker results is to chloroform all the survivors at the end of ten or fourteen days and make cultures from the blood and bile. A perfectly immunized animal is one that gives negative blood-cultures and at death presents no lesions particularly of the gall-bladder and gives negative cultures from the bile.

Throughout our experiments it has been our effort to kill our cultures of *B. typhosus* in a manner designed to obviate, so far as possible, the deteriorating effect of heat that has been evidenced by the work of numerous observers mentioned before. The introduction of heat, at all events for the purpose of obtaining sterile cultures, seems to us quite unnecessary, and, as one of us pointed out a number of years ago,[59] cultures of the dysentery bacillus may be sterilized and preserved by the simple addition of 0.5 per cent. trikresol or phenol, without destroying their toxic and antigenic properties to the same degree as in corresponding cultures that are first killed by heat at 60 C. (140 F.) and then preserved by trikresol. Very little attention appears to have been paid to this observation, although we believe that several have utilized the method with advantage, killing the cultures either with liquor formaldehydi or with trikresol. In the present series of experiments we have desired not only to kill the bacteria with little harm but also to collect and dry them in order that the dosage employed might be reckoned from weighed amounts of the dried bacilli, which had been previously ground in an agate mortar. For this purpose, as already stated in Experiment 1, we precipitate our various bacterial suspensions with equal parts of absolute alcohol, which accelerates the centrifugalization and the drying of the preparation. If the culture is to be sensitized, a definite amount of a potent, immune serum from the rabbit is previously added, the dosage being estimated in accordance with the agglutination titer of the serum in question and the number of bottles from which it was obtained, the amount being 1 c.c. of a serum that agglutinates the organism in question in a dilution of 1:20,000 to the suspended growth from each of the seven Blake bottles. After standing for two or three hours in an incubator at 37 C. (98.6 F.), the sensitized culture is left in the ice-chest over night, centrifugalized, the supernatant fluid removed, the sediment washed in the original amount of normal saline, recentrifugalized, and the volume restored to normal with sterile saline. This washed sensitized culture is then flocculated by adding an equal volume of absolute alcohol, dried, ground and weighed as in the unsensitized culture.

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[59] Gay: Vaccination and Serum Therapy Against the Bacillus of Dysentery, Univ. Penn. Med. Bull., 1902, xv, 307.

TABLE 2.—Summary of Experiment 4

Rabbit No.	Vaccination	Interval Before Inoculation Weeks	Agglutinins	Result
9	Plain Vaccine 1	12	1 : 100	Recovered
10	Plain Vaccine 1	12	Neg. 1 : 100	Carrier
12	Plain Vaccine 1	12	1 : 500	Carrier
13	Sensitized No. 1	12	Neg. 1 : 100	Recovered
14	Sensitized No. 1	12	Neg. 1 : 100	Recovered
Control 71	....	..	....	Carrier
Control 72	....	..	....	Carrier

We first compared the protective value of sensitized and unsensitized cultures. The results of our first experiment (Experiment 4) may be given, although few animals are involved, because the results were particularly clear-cut. Similar results will be shown in a later experiment in connection with other preparations.

Experiment 4.—Dried sensitized and unsensitized vaccines were prepared from the same growth of *B. typhosus* 5 (Army and Navy, Dorset strain) in the manner described above. Four rabbits were given 0.56 mg. of the dried unsensitized vaccine intravenously in 1 c.c. carbolated saline solution for three successive days. Two other rabbits were given the same amounts of the same vaccine *sensitized* in the same manner. One of the first series (plain vaccine) died by accidental injury. Three months after the last injection, the agglutination titer of the immunized rabbits was tested and they and two control animals of similar weights were given the standard test dose of living blood-agar typhoid bacilli (½ culture *B. typhosus* 3c) intravenously. The results are summarized in Table 2.

This experiment indicates the superior value of a sensitized vaccine over an unsensitized vaccine in producing a durable immunity. It further shows the unreliability of agglutinins as indicating resistance to actual infection with the typhoid bacillus. As we have already noted in the introduction, other observers have found that sensitized bacteria produce less agglutinins than untreated bacteria, but more tropic or sensitizing antibodies.

The method which we have described for preparing and grinding the vaccine is essentially similar to the one described by Besredka for the preparation of endotoxins, and it may, indeed, be shown in the case of the untreated vaccine that the supernatant fluid contains the greater part of the toxic substances of the whole vaccine (Besredka,[60] Steinitzer[61]). It seemed to us important to determine at this point whether or not the supernatant fluid of such a ground and suspended preparation of typhoid bacilli, or the sediment of bacterial bodies left on centrifugalizing this mixture contains the immunizing principle. In the following experiment (Experiment 5), in which the immunizing value of the two preparations is shown, it is very evident that it is the sediment or bacterial bodies deprived to a great extent of toxicity of the whole vaccine which is the essential immunizing principle. This immediately suggests the advisability, from the point of view of symptoms produced and of protecting value assured, of utilizing simply the remnants of the bacterial bodies obtained in such a vaccine rather than the whole vaccine itself. Experiments designed to test out the comparative value of the whole vaccine, on the one hand, and the sediment on the other, show very distinctly that the sediment possesses not merely as much but apparently actually more immunizing value than the whole vaccine (Experiment 6).

Experiment 5.—In this experiment are combined the results obtained from two different plain unsensitized vaccines. Vaccine 1, which has already been described (Experiment 4), and Vaccine 8 prepared in the same way but from blood-agar cultures grown on a blood-agar medium. The weighed, dried vaccine was in each case suspended in the proper amount of 0.5 per cent. carbolated saline solution and allowed to stand for forty-eight hours with thorough shaking at intervals. The suspended vaccine was then centrifugalized thoroughly, leaving an opalescent *supernatant* fluid (endotoxin) and a grayish white *sediment* of ground bacterial bodies. The supernatant fluid was removed and the sediment restored to the original volume by adding fresh carbolated saline solution. Rabbits were then vaccinated in series with the supernatant and sediment, respectively. The dosage of vaccine preparations given in three doses on successive days (volume 1 c.c. on each injection) and the resultant condition obtained on testing the animals with the standard dose of living culture is summarized in Table 3.

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[60] Besredka: Etudes sur le bacille typhique et le bacille de la peste, Ann. de l'Inst. Pasteur, 1905. xix, 477.

[61] Stenitzer: Ueber die Toxine (Endotoxine) der Typhusbazillen; Kraus and Levaditi, Handbuch der Immunitätsforschung, 1908, i, 193.

Table 3 shows the marked superiority of sediment over supernatant fluid in producing immunity from infection; all of the six sediment-treated animals recovered, whereas only one of eight supernatant animals failed to become carriers. The further unreliability of the agglutinin test is shown by the fact that in the first series the supernatant gives higher agglutinins but no protection.

TABLE 3.—Comparison of Immunizing Power of the Supernatant and Sediment Derived from Plain Typhoid Vaccines*

Rabbit No.	Vaccination Employed	Interval Before Testing Months	Agglutinins	Result
21	Supernatant of Vaccine 1. Total dosage, 1.68 mg. in three days	2	1: 50	Carrier
22	......	2	1:400	Carrier
26	......	2	1:200	Carrier
27	......	2	1:500	Carrier
23	Sediment of Vaccine 1; 1.68 mg. in three days	2	1:100	Recovered
28	......	2	1:400	Recovered
29	......	2	1:100	Recovered
		Weeks		
85	Supernatant of Vaccine 8; 10 mg. in three days	6	......	Carrier
86	......	6	......	Recovered
87	......	6	......	Carrier
88	......	6	......	Carrier
73	Sediment of Vaccine 8; 10 mg. in three days	6	......	Recovered
75	......	6	......	Recovered
76	......	6	......	Recovered

* The control rabbits are purposely omitted for simplification; they were positive, as is the rule.

One of our most recent experiments summarizes the facts that have been adduced as to the most protective preparation, and further brings out the fact, already stated, that the sediment actually protects better than the whole vaccine. In comparing the whole suspended vaccine with the sediment derived from such a vaccine, it should be noted that every technical error necessary to produce the sediment from the whole vaccine would tend to lessen the amount of material in the sediment, and the superior immunizing value of the latter is therefore so much the more striking. The results of this experiment are summarized in Table 4. The preparations used were prepared as already described, the preparations all being derived from an original suspension of *B. typhosus* 4 (recently isolated stain, October, 1913), grown on agar (Vaccine 20).

TABLE 4.—Comparison of Whole Suspended Vaccine with Sediment *

Rabbit No.	Vaccination Employed	Interval Before Testing Days	Result
262	0.5 mg. whole unsensitized Vaccine 20 in three days intravenously	32	Carrier
265	....	32	Recovered
266	....	32	Carrier
263	....	32	Died before testing
264	....	32	Died before testing
267	0.5 mg. sediment unsensitized Vaccine 20 in three days intravenously	32	Carrier
268	....	32	Recovered
269	....	32	Carrier
270	....	32	Recovered
271	....	32	Carrier
273	0.5 mg. whole sensitized Vaccine 20 in three days intravenously	32	Died acutely (anaphylaxis)
274	....	32	Killed by injury
275	....	32	Recovered
276	....	32	Recovered
277	0.5 mg. sediment sensitized Vaccine 20 in three days intravenously	32	Carrier (bile culture only positive)
278	....	32	Recovered
279	....	32	Recovered
280	....	32	Killed by accidental injury
281	....	32	Recovered

* Controls again purposely omitted.

The points which we have already brought out are emphasized in this experiment. Thus the percentages of recovery are 33 for whole unsensitized, 60 for whole unsensitized sediment, 66 for whole sensitized, and 75 for sensitized sediment. The first series also shows the greater toxicity of the whole culture as compared with its sediment.

We seem so far to have determined, then, that killed, sensitized cultures are somewhat more protective than plain, unsensitized, killed cultures of the typhoid bacillus and that the protection is due to the bacillary sediment and not to the supernatant endotoxic fluid. These sediments, moreover, seemed distinctly more protective than the whole vaccines from which they were derived.

In view of the assertions of Metchnikoff and Besredka, it remains to determine by our method whether or not living, sensitized cultures have any superior immunizing value over sensitized cultures killed by alcohol. For this purpose we have compared the living sensitized culture first with whole dried sensitized culture and then the living sensitized culture with the sensitized sediment. In these experiments the original bacterial suspension is sensitized, washed, and divided into two parts, one of which is left intact and the other precipitated by alcohol, dried, ground, weighed, and suspended in the original amount of fluid. Here again technical errors would militate against the immunizing strength of the dried culture, particularly when the latter is carried further to the sediment preparation. The results of two such experiments are summarized in Tables 5 and 6.

TABLE 5.—Comparison of Living Sensitized with Dried Whole Sensitized Vaccines

Rabbit No.	Vaccination Treatment	Interval Weeks	Result
171	1 mg. living sensitized culture* intravenously in three doses, on three successive days.	7	Recovered
172	....	7	Carrier
173	....	7	Carrier
174	....	7	Carrier
175	....	10	Recovered
176	....	10	Recovered
179	1 mg. dead sensitized culture intravenously in three doses, on three successive days.	7	Carrier
180	....	7	Recovered
181	....	7	Carrier
182	....	7	Carrier
184	....	10	Carrier
185	....	10	Recovered
186	....	10	Recovered

*Computed from same culture dried.

The first experiment shows that there is little or no difference to be shown between the immunizing powers of living sensitized cultures as compared with sensitized cultures killed by alcohol. The second experiment shows the immunization by dead sensitized sediment to be distinctly superior to that by living sensitized cultures.

PROTECTIVE VACCINATION IN HUMAN BEINGS BY MEANS OF THE MODIFIED SENSITIZED CULTURE

Our experimental results had reached a stage nearly a year ago when we felt justified in recommending the whole sensitized culture that we have described for use in protecting human beings against typhoid fever. It seemed demonstrated from the work on rabbits that the sensitized vaccine, washed, precipitated, killed with alcohol, and ground would protect at least as well and probably better than the ordinary vaccine that had not been sensitized, and we anticipated much fewer untoward symptoms following vaccination by the use of this sensitized vaccine. This absence of untoward effects has been fully justified in experience. Up to the present time over 1,000

TABLE 6.—Comparison of Living Sensitized Culture with Dead Sensitized Sediment *

Rabbit No.	Vaccination Treatment	Interval Weeks	Result	
296	1 mg. living sensitized culture intravenously in three doses on three successive days.	†		
289	....	7	Carrier	A
290	....	7	Carrier	
291	....	7	Recovered	
292	....	7	Carrier	
295	....	7	Carrier	
293	....	8	Carrier	
297	....	10	Recovered	
305	1 mg. dead sensitized sediment intravenously in three doses on three successive days.	†		
299	....	7	Recovered	B
300	....	7	Recovered	
302	....	7	Carrier	
303	....	7	Recovered	
304	....	7	Recovered	
298	....	8	Carrier	
301	....	9	Carrier	
306	....	9	Recovered	

* Of group A, two animals, or 25 per cent., recovered, and six animals, or 75 per cent., remained carriers. Of group B, five animals, or 62.5 per cent., recovered, and 3 animals, or 37.5 per cent., remained carriers.

† Died before testing.

students in the University of California have been immunized by Professor Force of the Department of Hygiene. The absence of reaction or the mildness of reaction has been very striking. Force[57] has already reported on the first 261 cases treated by this method, and less complete analysis of the subsequent cases agrees with the results obtained in the smaller number. In these 261 cases — apart from the local symptoms of induration, redness, and slight tenderness for one or two days—general symptoms, even of a mild grade, were very seldom observed. The vaccine was administered on alternate days, the treatment being completed within a week. The very fact that it was possible to give the second and third vaccinations on the second and fourth days after the first indicates strongly that even the local condition was so mild as to allow the subsequent inoculations. The dosage at first employed was $\frac{1}{16}$ mg. of dried culture suspended in 0.5 per cent. carbolated salt solution, corresponding, as we have determined, to 500 million bacteria. Since this produced little or no reaction, we have since increased it by one-half ($\frac{3}{32}$ mg.). In only six cases in the total number of inoculations practiced (671) did the temperature rise above 38 C. (about 100 F.). The symptoms were somewhat more pronounced in women than in men. In two cases of arrested tuberculosis the reaction was more marked and in a few cases that gave a history of previous typhoid fever the reaction was also slightly more pronounced. More recently we have employed the sensitized sediment vaccine in accordance with the experimental results that have just been obtained, which show conclusively that this substance is more immunizing than the whole sensitized preparation. This sensitized sediment gives even fewer reactions than the whole sensitized vaccine. Thus in a recent series that Force has vaccinated with sensitized sediment, using a dosage corresponding to 750 million bacteria, including 672 separate injections, malaise was noted in 1.8 per cent.; fever (above 99 F.) in 4.5 per cent.; pain in back in 2.4 per cent.; pain in head in 9 per cent., and pain in the arm in 3 per cent. In cards returned to us from 231 outside patients to whom the vaccine was distributed, 215 report total absence of local and general reactions (93 per cent.). Ten cases were marked as having shown generalized symptoms and three cases of typhoid recoveries and three patients who had been previously vaccinated also showed slight symptoms. These reactions may be compared with the following: Hartsock[62] obtained a mild reaction, by which he specifies "temperature up to 100 F. with slight general reaction, malaise, and considerable local tenderness," in 83 per cent. of men treated with U. S.

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[62] Hartsock, F. M: Antityphoid Vaccination, Jour. Am. Med. Assn., 1910, liv, 2123.

Army vaccine. Hatchel and Stoner[63] in 1,326 cases vaccinated with a polyvalent vaccine found malaise in over 58 per cent. and fever in 43 per cent. Albert and Mendenhall[64] used the U. S. Army strain in regular doses and intervals and found that the local reaction lasted for from three to five days. Fever as high as 103 F. was usual and malaise, headache, and insomnia were common.

Auché and Chevalier[65] have reported a notable failure of prophylactic typhoid vaccination in a person who received three injections in January, and in July came down with a well-defined case of typhoid fever. We agree with the authors that such cases should be reported as indicating the limitations of the method. Fornet has collected further cases in which the protection was not produced. In our early series of cases among the students treated with whole monovalent sensitized vaccine, in which it has been possible to follow the results with great accuracy, there has been one case of typhoid fever among the vaccinated persons five months after treatment; the recovery was uneventful. These instances go to prove that further perfecting of any method in vogue is necessary. Both Wassermann[24] and Vincent[15] have suggested the use of polyvalent vaccines; although the typhoid bacillus has been regarded as an unusually fixed species, the occurrence of minor biologic peculiarities, particularly in the fermentation of sugars, has been noted in the collection of organisms which we have studied. In accordance with these facts, as well as with the results that we have detailed, we have recommended a polyvalent sensitized sediment for prophylactic immunization against typhoid fever in human beings. The organisms, five in number, which have been used to produce the polyvalent mixture was isolated from recent cases of typhoid fever in the vicinity. An additional advantage in the use of sensitized over unsensitized cultures is that a strain of the typhoid bacillus may be used as in our polyvalent vaccine without producing any more symptoms than with a monovalent vaccine. It is particularly unnecessary to seek a "mild strain" as in the case of the U. S. Army vaccine. Our various strains are grown separately on Blake flasks, and the suspended cultures mixed and treated with a polyvalent immune serum obtained by immunizing rabbits with each of the strains in turn. This polyvalent sensitized sediment vaccine is now being manufactured and distributed free of cost by the California State Board of Health to any physicians in the state who may apply.

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[63] Hatchel, F. W., and Stoner, H. W.: Inoculation Against Typhoid, Jour. Am. Med. Assn., 1912, lix, 1364.

[64] Albert and Mendenhall: Reactions Induced by Antityphoid Vaccination, Am. Jour. Med. Sc., 1912, cxliii, 232.

[65] Auché and Chevalier: Un cas d'insuccès de la vaccination antityphique, Jour. de méd. de Bordeaux, 1913, xxiii, 371.

THE TYPHOIDIN REACTION

One of the greatest difficulties that has been present in determining the protective value of typhoid immunization as a whole has been the impossibility of determining the protection of a given group of persons by other means than the careful study of morbidity statistics among vaccinated people over a long period of years (Firth[66]). This difficulty delayed the final acceptance of typhoid immunization for at least eight years (1896-1904). Still less have we any means of determining whether or not a given person who has been vaccinated is actually protected against typhoid fever. We have already described in full the skin-test which Gay and Force[67] have employed as of presumptive value in testing resistance to typhoid infection.

A new summary of our results with the typhoidin may be of interest: In forty-four cases giving a definite history of typhoid fever from four and one-half months to forty-one years previously, forty have given a clear-cut skin-test to the concentrated growth of the bacillus. Of the four negative tests, two gave a strong skin-reaction to a similar preparation made from *B. paratyphosus A.* which we have found not to occur in anything like the same intensity in those cases that react to typhoidin. Only one of the remaining negative cases was further tested with the paratyphoidin solution and therefore only one of two cases was unexplainably negative and 95 per cent. (forty of forty-two) positive. We have a growing set of observations on comparative tests with two paratyphoidin solutions (A and B) in recovered typhoid and parathyphoid cases, and in those vaccinated against typhoid, which we may wish to present at a later time; they apparently show certain interesting group reactions.

Of the controls giving no history of typhoid fever, thirty-eight out of forty-four, or 86 per cent., have been clearly negative. Only five of these control cases (11 per cent.) reacted distinctly positively, and we believe may be explained as cases of aborted or mild, undiagnosed typhoid fever. This explanation is rendered probable by the observation, brought to our attention by Dr. Edward von Adelung, of a perfectly controlled clinical experiment on this subject. The observer, Dr. von Adelung, was a member of a family party of four which visited Germany nineteen years ago. About two weeks after drinking at a suspected water source, two of the members came down with a fever which ran the typical course of typhoid and was so diagnosed. The other two members of the party at the same time had mild symptoms, lasting one and three days, respectively, and consisting of headache, fever, malaise and a sensation of flushing, which they regarded as abortive typhoid. Neither of the latter two persons gave any other history of typhoid fever. When the skin-reaction was applied to the two cases that had run the typical typhoid course and to the two that had passed through the "abortive" attacks, all gave positive results. This well-controlled, natural experiment would seem to prove that the supposedly normal cases that react to typhoidin may well be those that have had abortive or undiagnosed typhoid fever in the past.

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[66] Firth: A Statistical Study of Antienteric Inoculation, Jour. Royal Army Med. Corps, 1911, xvi, 589.

[67] Gay, F. P., and Force, J. N.: A Skin Reaction Indicative of Immunity Against Typhoid Fever, Studies in Typhoid Immunization III, The Archives Int. Med., 1914, xiii, 471.

Our experience with the skin-reaction in those who have been vaccinated with various typhoid vaccines, including our own, has shown us that the majority of those who have been treated within a year and a half or two years may be expected to give a positive reaction, provided always that the last injection has been given at least a month previously. After two years the reaction is less likely to be positive. This experience with the skin-test would correspond very closely with what has been found clinically to be the usual duration of artificial typhoid immunity. We have felt justified, then, in recommending to students who have taken the typhoid vaccine previously that they should have a skin-test applied subsequently at intervals and that if it turned out to be negative they should repeat the treatment.

Since our last communication on the typhoidin test, we have been led to modify the preparations of the solutions employed. We found that some of the original preparation of typhoidin after four or five months failed to produce a positive reaction in cases in which we had been led to expect it. That this failure was due to a deterioration in the product was evidenced by subsequent positive results obtained in six such cases by means of a new preparation. Although such deterioration does not occur for a considerable time with tuberculin, it is known to take place in mallein and abortin. In the latter two cases and in tuberculin as well, better reactions have been obtained with preparations purified by alcohol precipitation and dried (compare Haring[68] and Meyer and Hardenbergh[69]). A similar preparation has been made by precipitating the original typhoidin with twenty volumes of alcohol, filtering, washing with absolute alcohol and ether, and then drying on porcelain plates over a vacuum with sulphuric acid. The control solution, 5 per cent. glycerin-bouillon evaporated to one-tenth volume, was treated in a similar manner. Ten c.c. of the original typhoidin yielded 0.78 gm. of dried powder when the culture had been grown for five days before evaporating. The dried powder from the same amount of a control solution gave only 0.5 gm. There is every reason to believe that this dried typhoidin will keep its potency undiminished for at least a considerable time. Our observations, however, extend only to its trial over a period of two months. We dissolve a small amount in carbolated saline equivalent to the original volume of concentrated typhoidin or even to a double concentrated solution and find that when kept in a cool place, it gives very good reaction in typhoid immunes for at least a month.

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[68] Haring, C. M., and Bell, R. M.: The Intradermal Test for Tuberculosis in Cattle and Hogs, Univ. Cal. Pub. Agric., 1914, Bull. 243, p. 94.

[69] Meyer and Hardenbergh: On the Value of the "Abortin" as a Diagnostic Agent for Infectious Abortion in Cattle, Jour. Infect. Dis., 1913, xiii, 351.

Our suggestion and growing belief that this skin reaction with typhoidin is a real measure of the protection that the person enjoys against typhoid fever is strengthened by observations on our immunized rabbits. We have already shown that the agglutinin titer is no indication of the resistance of a given animal to infection, and observations on the Widal reaction in man tend to the same belief (Ruediger and Hulbert[71]). The typhoidin reaction, on the other hand, is positive in that category of persons who are known to be protected against typhoid fever, namely, typhoid recoveries; it does not occur in persons who give no history of the disease, except in a small percentage that may reasonably be suspected of having had an abortive attack. The reaction further occurs in the majority of those persons who have been vaccinated against typhoid within the last two years and then gradually disappears. We had scarcely hoped to show differences in typhoidin reaction between incompletely and perfectly immunized rabbits as tested by our method of infection, which is, after all, a violent one, but our results in this respect have exceeded our anticipations. It is, however, easy to demonstrate fundamental differences between normal and immunized rabbits by the intradermal reactions. Cutaneous reactions were first tried, but abandoned as unsatisfactory.

With the intradermal reaction, performed by producing a tiny bleb under the skin by a short needle carrying typhoidin, a distinctive reaction is produced in rabbits that have been artificially immunized, but not in controls. In each case a patch on the abdominal surface is shaved the day before the test and two blebs produced under the epidermis by injecting in the one concentrated glycerin bouillon and in the other typhoidin. In the normal animal there is little or no difference between control and typhoidin spot. Both are usually surrounded by a red zone of a few millimeters, and there may be slight induration. In the animal that has previously been treated by typhoid vaccines there is a sharp difference between the two spots. The typhoidin spot, as early as five hours, but with increasing regularity and intensity to twenty-four hours, becomes surrounded by a red areola, is indurated, and quickly forms a firm, hard nodule with a yellowish, slightly softened center, from 2 to 5 mm. in diameter. This nodule persists at least for several days and may last two or three weeks. It is characterized histologically by an infiltration of lymphoid cells with which are mixed a few polymorphonuclear leukocytes.

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[71] Ruediger and Hulbert: Is Dried Blood as Reliable as Fresh Serum in Making the Widal Test? Am. Jour. Pub. Health, 1914, iv, 113.

Five normal rabbits tested in this manner gave negative reactions. Of thirty-five more or less perfectly immunized rabbits, twenty-six (74 per cent.) reacted positively. The differences in reaction between animals effectively immunized against our method of producing carriers and those which are not so fully protected is a relative matter when viewed in the aggregate, but in individual experiments is more striking. Thus, dividing our treated animals in respect to a negative or positive intradermal test and then comparing the results with their carrier condition or recovery on infecting the following day, we find that of nine with a negative intradermal test, seven, or 78 per cent., became carriers; of twenty-four with a positive intradermal test, thirteen, or 54 per cent., became carriers, a distinct, though only relative indication of agreement between the test and the absolute protection.

In Table 4 is given the comparative immunizing value of four different vaccine preparations. Before the test inoculation, the intradermal test was applied to these animals with the following results as compared with the eventual carrier conditions:

Group 1, vaccinated with whole unsensitized vaccine (three animals): intradermal test, 3 negative; carriers produced, 2; recovered, 1.

Group 2, vaccinated with unsensitized sediment (five animals): intradermal test, 4 positive; carriers produced, 3; recovered, 2.

Group 3, vaccinated with whole sensitized vaccine (three animals): intradermal test, 3 positive; acute death (anaphylaxis), 1; recovered, 2.

Group 4, vaccinated with sensitized sediment (four animals): intradermal test, 4 positive; carriers produced, 1; recovered, 3.

These results show a distinct relation between a positive intradermal test and recovery and between a negative intradermal test and establishment of the carrier state.

MECHANISM OF THE TYPHOIDIN REACTION

We do not intend, at this time, to discuss fully the experimental evidence and the hypotheses that have been brought forth to explain the generalized and localized reactions that follow the injection or application of bacterial extractives like typhoidin in infected or immunized individuals. Of these reactions, the one to tuberculin has naturally been most studied. Some of the most interesting and debatable questions that seem to have arisen in connection with the tuberculin reaction are, first, whether or not it is in reality a reaction exactly similar to the anaphylactic reaction with serum, and second, whether it is due to an interaction of antigen and antibody accompanied, it may be, by alexin fixation (Wassermann and Bruck). As regards the local, cutaneous test we have the further possibility that the antibodies, hypothetically furnished to unite with the antigen, may be furnished from the general circulation or due to local cellular response (von Pirquet). There is certainly evidence in the case of typhoid recoveries that the protection is cellular rather than humoral, and the relative inefficiency and short duration of artificial vaccination against typhoid fever may well be due to the fact that the immunity is not sufficiently cellular in type.

It seemed possible to attack certain of these problems in relation to the localized typhoidin test. The first question that arose was whether or not the reaction was due to the local combination of antigen and antibody. As has already been shown, the sensitized vaccine which we have employed gives less reaction, both general and local, than the untreated vaccine when used in the prophylactic treatment of human beings. We should not, therefore, expect that the local application of a sensitized vaccine in normal individuals would call forth any, or at any rate, not so marked a response as the untreated vaccine. And yet, it was argued, if the local typhoidin reaction in an immunized individual is really due to a combination of antigen and antibody, sensitized vaccine, which is just such a combination, might produce a local reaction in a normal individual.

Experiment 6.—Suspensions of unsensitized, dried Typhoid Vaccine 4 and of sensitized, dried Typhoid Vaccine B were made in the proportions of 5 mg. of vaccine to 1 c.c. of carbolated saline solution.

On normal rabbit 319 were produced intradermally small blebs of the foregoing sensitized and unsensitized suspensions. Twenty hours later the following condition was observed:

"Vaccine 20 bleb (unsensitized) measures 3 by 5 mm., is not markedly raised, and has no yellowish center.

"Vaccine B bleb (sensitized) measures 6 by 5 mm., is surrounded by an areola that is indurated, markedly higher than the other point, and with large, firm, raised yellowish papule, which resembles in all respects the positive skin-reaction obtained in immunized animals except that the surrounding areola is not quite so extensive."

Normal Rabbit 322, treated in the same manner, showed slightly more reaction about the unsensitized spot than the sensitized, but no distinct papule was present as in Rabbit 319.

A skin test with the two preparations was then tried on two normal, unvaccinated human beings. No reaction was noted with either spot. Since the intradermal test is recognized as being more sensitive, it was next tried on normal persons.

Thus, when comparative intradermal tests with sensitized and unsensitized vaccine were made on two normal rabbits, one of the two showed a much more marked reaction to the sensitized vaccine than to the unsensitized, the sensitized vaccine reaction resembling in all respects that produced in an immunized animal by the injection of typhoidin.

Of four human beings that were similarly tested by intradermal blebs, three showed a very characteristically increased reaction to the sensitized vaccine over the unsensitized vaccine. In only one case were the reactions produced by the two suspensions similar, and both were so marked as to suggest the possibility of an overlooked typhoid history. We regard this experiment, then, as distinctly indicating that the local reaction to typhoidin is due to an interaction of antigen and antibody.

TABLE 7.—Normal Persons Tested by Intradermal Test with Sensitized and Unsensitized Typhoid Vaccines*

No.	Reaction to Sensitized Vaccine (5 mg. to 1 c.c.)	Reaction to Unsensitized Vaccine (5 mg. to 1 c.c.)
1	24°. Large indurated area 80 by 100 mm.	Same as other bleb
2	18°. Red area 50 by 58 mm. (Foregoing solutions diluted one-tenth concentration) (0.5 mg. to 1 c.c.)	2.2 by 2.2 mm. Less red
3	24°. Demarcated, red, slightly indurated·area, painful 7.1 by 5.4 mm.	Indefinite, slightly red area, 4.2 by 2.1
4	24°. Red, demarcated area 6.2 by 5.2 mm.	Less red, not demarcated, 4.9 mm.

* The tests were applied by making blebs, one on each upper arm.

One further point on the mechanism of the typhoidin skin-test has been brought out by experiments on rabbits. Having reached a logical assumption that the skin-test is due to the interaction of antigen and antibody in the immune individual, it remained to show whether the antibody furnished for this combination comes from the circulating blood or is due to local tissue reaction. If the first hypothesis is true, immune serum should transfer the reactionability to a normal animal and an immune animal that reacts should at least partially lose this power if exsanguinated and transfused with normal rabbit blood. Both these conditions were found possible of realization.

Experiment 7.—The results of this experiment may best be described in parallel columns (Table 7).

This experiment, as well as another one carried out under the same conditions and with the same results, shows clearly that the abstraction of blood from an immune animal that reacts strongly to an intradermal test of typhoidin renders it almost completely insusceptible, whereas a control immune reacts a second time on corresponding days. The blood of the immune animal transfers the susceptibility to the typhoidin reaction to a normal animal, which has previously reacted negatively. We may conclude, therefore, that in the case of immunized rabbits, and perhaps also in the case of human beings artificially immunized against typhoid fever, the skin-reaction may be accounted for by the interaction of the antigen with antibodies in the circulating blood. We do not wish at all to suggest that the skin-reaction in typhoid recoveries is due to the same mechanism. The absence of demonstrable antibodies in the majority of these cases, as well as their superior type of immunity, would lead one to think that the skin reaction may be cellular rather than humoral in character.

TABLE 8.—Transfer of Reactibility to Skin Reaction by Typhoidin

Date	Typhoid Immune Rabbit 298	Typhoid Immune Rabbit 293	Normal Rabbit 329
April 7...	Intradermal test applied	Intradermal test applied	Induration test applied
April 8...	Reaction strongly positive	Reaction strongly positive	Reaction negative
April 9...	....	Bled for 50 c.c. and rapidly transfused from a normal rabbit	
April 10...	Intradermal test applied	Intradermal test applied	Given 20 c.c. serum from Rabbit 293
April 11...	Reaction strongly positive	Reaction negative; slight induration; no papule	Intradermal test applied
April 12...	....	....	Reaction distinctly positive and increasingly so to seventy-two hours; induration; papule and yellow center.

SUMMARY AND CONCLUSIONS

The history of the development of artificial immunization against the typhoid bacillus in animals and human beings is reviewed, and some of the many preparations of the typhoid bacillus used for this purpose are discussed. Particular attention is drawn to the reputed advantages of living sensitized typhoid vaccine (Besredka) as opposed to other types of vaccine. The many vaccines that are still being advocated indicate that the best vaccine has not yet been found and that the best method of proving which is the best vaccine has not been determined.

In the present article we explain again the method of testing typhoid immunity by means of an artificial typhoid-carrier state in the rabbit, and again advocate it as the best test for this purpose short of experimentation on anthropoid apes. We find this method admirably suited to indicate slight differences in typhoid preparations as regards their ability to produce an immunity against a subsequent typhoid carrier condition produced by intravenous injection of living typhoid bacilli. In successive series of experiments we have been able to show by this method, first, that untreated bacteria, killed and precipitated by alcohol, dried, ground and weighed, do not protect so well as typhoid bacilli that have been sensitized by an immune serum and subsequently treated in the same manner. Second, it has been possible to show in the case of the unsensitized, dried bacteria that the sediment of bacterial bodies freed from the supernatant, endotoxic fluid as prepared from these dried cultures contains the immunizing principle almost in its entirety. The sediment of either sensitized or unsensitized cultures protects not only better than the supernatant fluid from these sediments, but actually better than the whole, unseparated mixture. Third, we find that alcohol-killed, sensitized cultures protect almost as well as living, sensitized cultures and that the sediment of alcohol-killed, sensitized cultures protects better than living, sensitized cultures.

Sensitized cultures of the typhoid bacillus, whether whole or as sediment, produce little or no reaction in human beings with the possible exception of those who have previously suffered from typhoid fever or have been immunized. By comparing our results with those of certain other observers, we conclude that a considerable degree of reaction, both local and general, is avoided by the use of these sensitized cultures, which possess the further advantage, so far as our experimental work can determine, of producing a more durable type of immunity.

We recommend vaccination at short intervals (two days) in human beings, which is rendered quite possible with the mild vaccine we employ, and is evidenced from animal experimentation, as giving rise to less toxic effect and to fully as durable an immunity as vaccination at longer intervals. This type of vaccination has the further advantage of completing the prophylactic treatment of three injections within a week.

We further believe that a polyvalent vaccine derived from strains of the typhoid bacillus isolated in the vicinity of cases that are to be treated is advantageous, judging largely from the work of other observers. A further advantage in the use of sensitized cultures is that a polyvalent vaccine, no matter how recently the strains may have been isolated, is also almost entirely free from untoward effect. We have no direct experimental evidence bearing on the superior immunizing value of such a polyvalent vaccine. The dosage of vaccines in human beings which we recommend is empirical and based on the usage of other experimenters.

We recommend, then, for prophylactic immunization against typhoid fever, three injections of the sediment of a dried, ground, sensitized culture of several local strains of the typhoid bacillus mixed together, given at two-day intervals and in a dosage of $3/32$ mg. of the original dried culture, which corresponds, as has been determined, to a dosage of approximately 750 million living typhoid bacilli.

Our own experimental and clinical results, as well as the work of many other investigators, leads us to regard the agglutination reaction with the serum of immunized animals and human beings as by no means indicative of the degree of protection afforded against infection with the typhoid bacillus. Of far more prognostic significance is the skin test with the typhoidin solution, which Gay and Force have recently described. Further summaries of the results with this test show that this reaction is positive in the majority of recovered cases of typhoid (95 per cent.), even as far back as forty-one years, and that it occurs in only about 11 per cent. of persons who give no history of typhoid or of typhoid immunization. Distinct evidence is brought forth that indicates that these supposed normals reacting to typhoidin have suffered from an abortive or undiagnosed typhoid fever. Persons immunized with various types of typhoid vaccine react in the majority of cases for about two years and then become more frequently negative. We regard a negative skin-test after vaccination as indication for revaccination.

That a positive typhoidin test bears a distinct relation to protection against typhoid fever is further indicated by similar reactions produced intradermally in our immunized rabbits. In spite of our more violent method of artificial infection as against natural infection in typhoid fever, we find that animals that resist becoming carriers show a higher percentage of positive intradermal tests to typhoidin administered the day before; whereas those that are not immunized sufficiently to resist the carrier state more frequently show a negative typhoidin reaction.

We suggest the use of dried, alcohol precipitate from typhoidin solution as a stock from which the test solution may be prepared, since it has been found that the original typhoidin solution deteriorates within a few months.

Experiments dealing with the mechanism of the local typhoidin reaction indicate that it is due to an interaction of antigen and antibody, as is shown by the fact that the *intradermal* test of a sensitized vaccine will produce a characteristic reaction in human beings, whereas the corresponding amount of an unsensitized vaccine produced no such reaction. The paradox of producing a more severe local reaction with the sensitized vaccine, which has been found to produce less violent reactions when used for immunization, is only apparent. Further experiments with rabbits indicate that in the condition of artificial immunization against the typhoid bacillus, the antibodies which unite with the antigen to produce the local test are in the circulating blood, as is indicated by the passive transfer to a normal rabbit by means of serum from an immune rabbit of susceptibility to this reaction. This is further evidenced by the extraction of blood from the immunized rabbit and a corresponding loss of reaction. We do not regard this experiment, however, as indicating the circulatory nature of the antibodies in the recovered typhoid persons who almost invariably react to typhoidin.

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A CASE OF DIABETES INSIPIDUS *

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Johann Peter Frank[1] in 1794 first gave a definition for diabetes insipidus when he described it as a long-continued abnormally increased secretion of non-saccharine urine which is not caused by a diseased condition of the kidneys. Thomas Willis,[2] however, had previously recognized a distinction between a saccharine and a non-saccharine diabetes by discovering the fact that, in certain cases of polyuria, the urine had a sweet taste. The two first cases of diabetes insipidus, reports of which were collected and published in English, were recorded by Dr. Simmons[3] two years before Frank's work appeared, and are of such historical importance that it is of interest to quote them in some detail.

AN ACCOUNT OF TWO CASES OF POLYDIPSIA, OR EXCESSIVE THIRST

"Almost all the modern nosologists have introduced into their systems a disease, to which, on account of the excessive thirst that forms its characteristic symptom, they gave the name of polydipsia; but in none of the examples of it they have been able to collect does it appear to have been idiopathic; and Dr. Cullen expressly says that it is almost always symptomatic. A very curious instance, however, of such an affection, apparently depending on a peculiarity of temperament, or what is called idiosyncracy, occurs in a woman now living in Paris, of whose case the first account was given by M. Bessejon de la Chassagne, in a letter which we shall here translate from the Paris *Journal* of May 1, 1789."

"PARIS, April 18, 1789.

"To the Authors of the Journal,

"Gentlemen:—You will interest equally the humanity and curiosity of the public, by inserting in your Journal the following anecdote, which, without doubt, will be considered as a phenomenon. I have assured myself, with the most scrupulous accuracy, of the facts which I announce, but I leave to the reader to explain their nature and cause.

"Catherine Bonsergent has been remarked from her tenderest years. A burning thirst, a drought without example, with which she has been con-

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* Submitted for publication May 19, 1914.

* From the Medical Clinic of the Peter Bent Brigham Hospital, Boston.

* Reported at a meeting of the Boston Society of Medical Sciences, April 14, 1914.

1. Frank, Johann Peter: De Curandis Hominum Morbis, iii, Class V (Profluvia), Ordo I (prfl. serosa), Genus II (diabetes), p. 28, Florence, Coen et Socios, 1832.

2. Willis, Thomas: De Diuresinimia, ejusque remedio, et speciatim de Diabetes, in cujus Theoriam et Therapiam inquiritur, Opera Omnia, Pharmaceutice Rationalis, Sectio IV, Caput III, Henricum Wetstenium, Amsterdam, 1682.

3. Simmons: Medical Facts and Observations, London, J. Johnson, 1792, ii, 73.

tinually afflicted from the time of her birth, have always fixed on her the attention of persons of observation. Her parents, after having entrusted the first care of her to a nurse, took her home when she was three years old. It was not long before they observed that an extraordinary quantity of water was consumed in the house, and at length discovered that their daughter drank, every day, to the amount of two pailfuls. In summer she drank the first water she could meet with, in winter she had recourse to snow and ice, and she was always careful to reserve as much as would enable her to drink abundantly during the night.

"At the age of twenty-two years she married one Fery, a cobbler, from whom she contrived to conceal her complaint till after their marriage. She has had by him eight children, three of whom are still alive, and she is now pregnant with a ninth. What appears particularly extraordinary is, that during her lyings-in instead of having recourse to such food and liquors as would seem most likely to strengthen her, she chooses rather, for the sake of satisfying her thirst, which at those times is more intense, to drink almost without interruption, three or four quarts of coldest water. During the severe cold of last winter this woman, who was then pregnant, drank to the amount of four pailfuls of water in twenty-four hours; and her husband being unable to afford the expense of such a consumption, was under the necessity of supplying her with melted snow and ice. The price of a load (two pailfuls) of water, at that time, was six sols; and the quantity she required would have cost him more than he earned by his work.

"This woman has never made use of any sort of strong liquors, and if she drinks only a single glass of wine she feels an uneasy sensation in all her limbs, and seems to be in danger of fainting. She is not dropsical; she even enjoys a pretty good state of health; she voids naturally all the water she drinks, but her urine is extraordinarily foetid. She lives at the Hôtel des Arts, Fauxbourg Saint Martin.

"Signed Bessejon de La Chassagne.
"Prêtre de St. Laurent."

"While the preceding account was preparing for the press the following paragraph appeared in the Lincoln *Mercury* of Friday, December 9, 1791.

"'However extraordinary the following circumstances may appear, it may be depended on as fact: A man who lives with Mr. John Julyan, of Woodstone, near Peterborough, is afflicted with such an immoderate degree of thirst as obliges him to drink the astonishing quantity of three gallons of water a night, and one gallon a day; and what makes this appear still more extraordinary, he has continued this practice for twenty-three years.'

"The facts described in this paragraph bore too striking a resemblance to those he had just before received from Paris not to excite in the editor a wish to see the case more fully and satisfactorily investigated. This has since been done through the kind offices of Sir John Banks, Bart., who being acquainted with a gentleman in the neighborhood of the patient, on whose accuracy he knew he could depend, had the goodness to trasmit to him some queries from the editor relative to this subject, with a request that he would engage in the inquiry.

"In consequence of this request, Mr. Maxwell, the gentleman alluded to, sent for the man to his house, where he remained a whole night, and was carefully attended to. The result of this investigation, which sufficiently established the truth of the facts, we shall here give in Mr. Maxwell's own words.

"Extract of a letter from Mr. George Maxwell to Dr. Simmons, dated Fletton Lodge, near Peterborough, Dec. 18, 1791.

"With respect to the water drinker, who is the subject of your inquiry, and who lives at Stanground, near Woodstone, though he works at the latter place, it happens that Mr. Beal, the person who now looks after my farm, employed him as a thrasher more than twenty years ago. His account of this man is, that he always drank the quantity he is now said to do, or at least was at that time reputed to drink it.

"As he resided three or four miles from Mr. Beal's habitation, the latter used to make up a bed for him in his house, and Mr. Beal observed that at night he always took a bucketful of water upstairs with him.

"I have a laborer likewise who has worked with him, and who says in mowing time this man always takes four quarts of water out with him from a pump in the village, besides two quarts of beer.

"The man adds, that he has consulted several medical gentlemen about his complaint, but has not been able to get anything that could, in the least, relieve him."

Diabetes insipidus, notwithstanding the long time it has been recognized as a disease, is uncommon. Eichorst[4] saw only 17 cases out of 35,942 patients in the medical clinic at Zürich between 1876-1877. Gerhardt[5] found 55 cases among 113,600 treated at the Charité, Berlin, from 1877 to 1896; and Futcher[6] 7 cases out of 403,535 patients treated in the Johns Hopkins Hospital and Dispensary from 1889 to 1904. Thus from a total of 553,077 cases, there are only 79 of diabetes insipidus, or 14 cases per hundred thousand.

Out of 133 cases of the disease selected from his own observations and the literature up to 1892, Stoermer[7] found that diabetes insipidus affected men more often than women, and that it was commoner in the period of life before forty than later. In children also, Külz[8] showed that boys were more commonly affected than girls. It has a tendency to affect certain families, as stated by Clay,[9] Gee,[10] Lancereaux,[11] and most notably by Weil,[12] who reports seventeen cases in one family during three generations; it is chronic, but is usually not fatal on its own account.

The cases which have come to necropsy have shown various pathological conditions. The most common anatomical lesion has been brain tumor of either a malignant or of an inflammatory nature. The symptoms occur most frequently in association with tumors which involve the floor of the fourth ventricle or are in its immediate vicinity, or which are in the hypophyseal gland. On the other hand, as shown by Simmonds,[13] tumors occur in this situation without establishing the symptom-complex.

In the literature, repeated reference is made to the importance of either congenital or acquired syphilis as an etiological factor. In such cases the underlying process is usually either a gumma in the floor of the fourth ventricle or in the hypophysis, a basilar meningitis, or an endarteritis with connective tissue proliferation. A certain number of cases have been reported in which no demonstrable anatomical changes existed.

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[4] Eichorst: Cor.-Bl. f. schweiz. Aerzte, 1888, xviii, 393.

[5] Gerhardt: Nothnagel's Specielle Pathologie und Therapie, 1900, vii, 7.

[6] Futcher: Tr. Assn. Am. Phys., 1904, xix, 247.

[7] Stoermer: Inaug. Diss., Kiel, 1892.

[8] Külz: Handbuch der Kinderkrankheiten, 1878, iii, Ersste Hälfte, p. 285.

[9] Clay: Lancet, London, 1889, lxvii, part 1, p. 1188.

[10] Gee: Saint Bartholomew's Hosp. Rep., 1877, xiii, 79.

[11] Lancereaux: Thèse de Paris, 1869.

[12] Weil: Virchow's Arch. f. path. Anat., 1884, cxv, 70.

[13] Simmonds: München. med. Wchnschr., 1914, lxi, 27.

These facts have led to the classification of diabetes insipidus into two types:

1. Symptomatic, including the cases in which there is evidence of organic disease either in the brain or elsewhere, the lesion being considered the cause of the diabetic symptoms.
2. Primary or idiopathic cases, including those in which there is no evident organic basis for the disease.

The part that the kidney plays in causing the characteristic symptoms of diabetes insipidus has been studied from various points of view. Claude Bernard,[14] Eckhard,[15] and more recently Kahler,[16] have attacked the problem of the physiology of polyuria by animal experiments. Their results in brief show that wounds of the floor of the fourth ventricle frequently produced polyuria, but that in rabbits, at least, there was no definite point which would produce a pure "hydruria." Section of the spinal cord at the level of the sixth or seventh cervical vertebra, as well as at higher levels, produced an immediate and persistent suppression of urine. Sections below those levels gave less certain and less prompt results, whereas sections of the cord below the twelfth dorsal vertebra had practically no effect whatever on the secretion of the urine. Schäfer and Herring[17] have studied the action of the secretion of the hypophysis. They found that a systemic rise in the blood-pressure was produced by intravenous injections of the gland extract, with a specific dilating effect on the renal blood-vessels. They believed that pituitary extract had a pharmacological action on the kidney analogous to that of caffein.

It may be concluded, therefore, that the nerve fibers which supply the kidneys pass from the medulla to the level of the sixth or seventh cervical vertebra, where they leave the cord in the uppermost thoracic nerves and pass along the wall of the aorta and renal arteries to the kidneys; and that an experimentally produced polyuria is an irritation phenomenon or is due to an increased amount of the secretion of the pituitary gland in the circulating blood.

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14. Bernard: Leçons sur les propriétés physiologiques et les altérations pathologiques des liquides de l'organisme, Paris, 1858, 1, 397.
15. Eckhard: Beiträge zur Anatomie u. Physiologie, 1869, iv, 1, 153; 1870, v, 147; 1872, vi, 51.
16. Kahler: Ztschr. f. Heilk., 1886, vii, 105.
17. Schäfer and Herring: Phil. Tr. Roy. Soc., London, 1906, Series B, cxcix, 1.

Clinical studies on the action of the kidney in diabetes insipidus have been made by many observers with varying interpretations. Erich Meyer,[18] Tallqvist,[19] Socin[20] and others believed that the disease was caused by a primary inability of the kidney to excrete urine of normal concentration. Therefore the urine was dilute, of low specific gravity, and of low molecular concentration as estimated by the freezing-point. When sodium chlorid or nitrogen was added to the diet, these substances were excreted by a marked polyuria, or were compensated for by the retention of other substances. Theocin did not act as a diuretic, but stimulated the concentrative powers of the kidney for a short time, so that during its administration an increased amount of chlorid was excreted, which was later retained after the drug was omitted. until the chlorid balance was regained. Finkelnburg,[21] by making observations at frequent intervals felt that the concentrative power of the kidney was not lost, agreeing with Schwenkenbecher[22] and Forschbach and Weber,[23] who considered diabetes insipidus a disease in which the kidney under pathological conditions of stimulation excreted abnormally large amounts of urine. Frank[24] observed a case due to a bullet which lodged in the pituitary fossa. From a review of the literature and this observation, Frank thought that many cases of essential diabetes insipidus could be explained by hyperfunction of the pituitary gland with a resultant diuretic action. To meet the argument that an increased blood-pressure and other pharmacological activities of this substance were not observed, he replied that smooth muscle had an antagonistic action against pituitary extract, while its diuretic effect was unhindered. Cushing's[25] observations emphasized the importance which this gland plays in producing the disease. Ebstein,[26] finally, considered that the condition was due to a primary polydypsia with resultant polyuria because if the kidneys were primarily at fault, when the fluid intake was diminished, the specific gravity would not increase, whereas in many of the observations recorded, the opposite has taken place and the concentration and specific gravity has risen as in normal individuals.

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[18] Meyer: Deutsch. Arch. f. Klin. Med., lxxxiii, 1; Deutsch. Klin., 1910, xiii, Ergnzgsbd., ii, 271; Ztschr. f. klin. Med., 1912, lxxiv, 352.

[19] Tallqvist: Ztschr. f. klin. Med., 1903, xlix, 181.

[20] Socin: Ztschr. f. klin. Med., 1913, lxxviii, 294.

[21] Finkelnburg: Deutsch. Arch. f. klin. Med., 1910, c, 33.

[22] Schwenkenbecher: München. med. Wchnschr., 1909, lvi, 2564.

[23] Forschbach and Weber: Ztschr. f. klin. Med., 1911, lxxiii, 221; Forschbach: Ztschr. f. klin. Med., 1913, lxxvii, 153.

[24] Frank: Berl. klin. Wchnschr., 1912, xlix, 393.

[25] Cushing: Med. Communications, Mass. Med. Soc., 1913, xxiv.

[26] Ebstein: Deutsch. Arch. f. klin. Med., 1909, xcv, 1.

On the whole, from the literature it appears that diabetes insipidus is usually of the symptomatic type, though it may be idiopathic. The exact cause of the polydipsia and polyuria is undetermined; certain observers believe that the kidney's concentrative powers are at fault, others that the polyuria results from abnormal thirst, and others that the kidney is under a pathological condition of stimulation which may be due to an increased internal secretion of the hypophysis with resultant polyuria and polydipsia.

REPORT OF CASE

The following case is reported on account of the rarity of the condition and because it seems possible that studies in renal function according to present methods may be of interest in this disease.

Alfred L. (Peter Bent Brigham Hospital Medical No. 911) of Syrian descent, 12 years old, white, a schoolboy, born in Jamaica, W. I., entered the hospital with the following history:

His father is living and well; he has four brothers and one sister who are living and well. His mother is dead, but the patient is unable to state from what cause. To his knowledge, there is no family history of polyuria or polydipsia.

He has lived in Boston as long as he can remember. In 1909 (five years ago) he had measles, scarlet fever and chicken-pox. Otherwise he has always been well.

He is in the fifth grade in school. His appetite is not abnormal, his digestion is good, his bowels are constipated so that they move only twice a week. His eyesight is good. He has had no headache. He drinks two or three cups of coffee daily, but no tea.

Five months ago he first noticed that he had to get up at night to urinate. Since then he has complained of an increasing thirst and frequency of micturition, so that at present he urinates about ten times a day, and four or five times at night, passing more than a pint each time. He has not lost weight or strength, and has no pain with micturition.

He is a fairly well-nourished boy, of normal skeletal and sexual development. There are small glands palpable in the neck, and in the epitrochlear and inguinal regions. The ears, nose, mouth and throat are not remarkable. The heart is not enlarged to percussion; no murmurs are heard, and the aortic second sound is normal. The systolic blood-pressure is 106; the diastolic is 70. The lungs are resonant throughout; breath and voice sounds are not remarkable; no râles are heard. The abdomen is negative.

Neurological examination reveals nothing abnormal. The fundi oculorum are negative, no contraction in the form field of either eye is demonstrable, but determination of the color fields is unsatisfactory because of the patient's lack of cooperation.

An x-ray of both kidneys, both ureters and the bladder shows no abnormal shadows. An x-ray of the skull shows rather marked cerebral impressions, but is otherwise negative. The sella tursica shows normal size and shape. Its largest diameter is about 10 mm., and it is about 7 mm. deep. The anterior and posterior clinoid processes are distinct. The development of the skeleton of both hands corresponds to the age.

Examination of the patient's blood shows a white count of 12,000, hemoglobin of 75 per cent. Smear shows slight achromia and anisocytosis of the red corpuscles. A differential count of the white cells gives 61 per cent. of neutrophils; 31 per cent. of small mononuclear cells and 3 per cent. of large

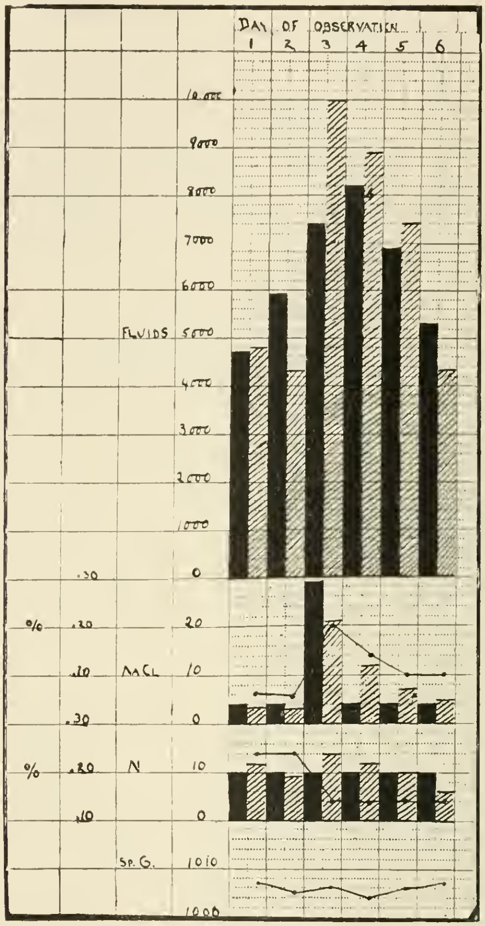

Chart 1.—Tests for renal function. One c.c. of phenolsulphonephthalein solution was injected intramuscularly, and the excretion at the end of one hour and two hours was determined colorimetrically (30 per cent. for one hour, 60 per cent. for two hours) according to Rowntree and Geraghty (The Archives Int. Med., 1912, ix, 284). Potassium iodid, 0.5 gm., was given by mouth, and the length of its excretion time (twenty-four hours) found by Sandow's test. Lactose, 2 gm. in 20 c.c. of distilled water, was injected intravenously and its elimination followed both qualitatively by Nylander's test, and quantitatively by polarimetry (four-hour excretion, 60 per cent. recovered). The diastase content of the urine was estimated (1 c.c. urine digests 7 c.c. of 0.1 per cent. starch solution in half an hour) by Wohlgemuth's method (Wohlgemuth and Noguchi: *Berl. klin. Wchnschr.*, 1912, xxiii, 1069). The blood nitrogen was estimated (17.5 mg. per 100 c.c. of blood) by Folin and Denis' micromethod (*Jour. Biol. Chem.*, 1912, xi, 527). The chlorid determinations were made by Volhard's method, and the nitrogen determinations by Kjeldahl's method. In this and the following charts, unless otherwise stated, the solid black column represents intake of fluid, sodium chlorid or nitrogen, and the cross-hatched column output. The dotted line represents concentration of chlorid or nitrogen per hundred c.c. of urine, and specific gravity.

mononuclear cells. The patient's stool is well formed, normal, contains no occult or free blood, and no ova.

The Wassermann reaction of the patient's blood-serum is strongly positive. The spinal fluid, however, is under normal pressure, contains 2 cells per cubic millimeter, and reduces Fehling's solution. The globulin and Wassermann reactions are negative.

While under observation the patient has passed from 4 to 10 liters of urine a day with a correspondingly large fluid intake. Repeated examinations of the urine have shown a low specific gravity, no albumin or sugar, and no blood, pus or casts in the centrifuged sediment.

On the whole the history is characteristic of diabetes insipidus. The only positive clinical finding is a Wassermann reaction in the blood-serum. Since the cerebrospinal fluid is normal, there is no evidence of any meningitis. Thus the case clinically must be considered as an idiopathic diabetes insipidus, or, if symptomatic, due to a small lesion in the brain, possibly of a syphilitic nature, without any focal manifestations.

Various observations have been made on the patient's renal functions which are discussed with illustrative charts. During the entire period, unless otherwise stated, the patient has been given daily as much water as he chose, and has eaten a constant diet containing 10 gm. of nitrogen and 4 gm. of sodium chlorid in such form as to yield a total of 2,000 calories.

Schlayer[27] and his co-workers have assumed that different portions of the renal structure have selective excretory activities which can be tested individually. Experiments have shown that water and lactose, when injected intravenously, are put out mainly through the action of the glomerular vessels, while sodium chlorid and potassium iodid are concentrated in the tubules. Normally large amounts of salt are put out by one of two methods. If the sodium chlorid is given without extra water it is excreted by increased concentration in the urine; if given with additional water it is excreted partially by increased concentration and partially by an increased amount of urine. In severe tubular injury with a normal vascular system, salt is retained in the body, since the tubules are impermeable, and potassium iodid is excreted over a long period of time. When the glomerular function is abnormal, the excretion of water is interfered with. There may be polyuria due to a hypersensitive condition of the blood-vessels by which they respond continuously to the small amount of diuretic substances contained in food, and exaggerated by the addition of sodium chlorid; or there may be oliguria due to a torpid condition of these vessels as a result of which they are unable to excrete the usual amount of fluid. Urine of low specific gravity, therefore, is of two sorts. A "tubular hyposthenuria" occurs when the renal vessels are not hypersensitive, and the tubules have lost their concentrative powers. "Vascular hyposthenuria" is found when the vessels are hypersensitive and respond with polyuria to the diuretic properties of sodium chlorid. Between these two extreme types are various gradations.

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[27] Schlayer: Deutsch. Arch. f. klin. Med., 1911, ci, 333; 1911, cii, 311.

In our case, according to these premises, there existed a constant "hyposthenuria." The total renal function was not affected, since the phthalein elimination was normal, and there was no accumulation of nitrogen in the blood. The diastase in the urine was low, but was not entirely lacking, agreeing with the observations of Benczur[28] and Geyelin,[29] and opposed to Strauss[30] and Rosenthal,[31] who found it absent in this condition.

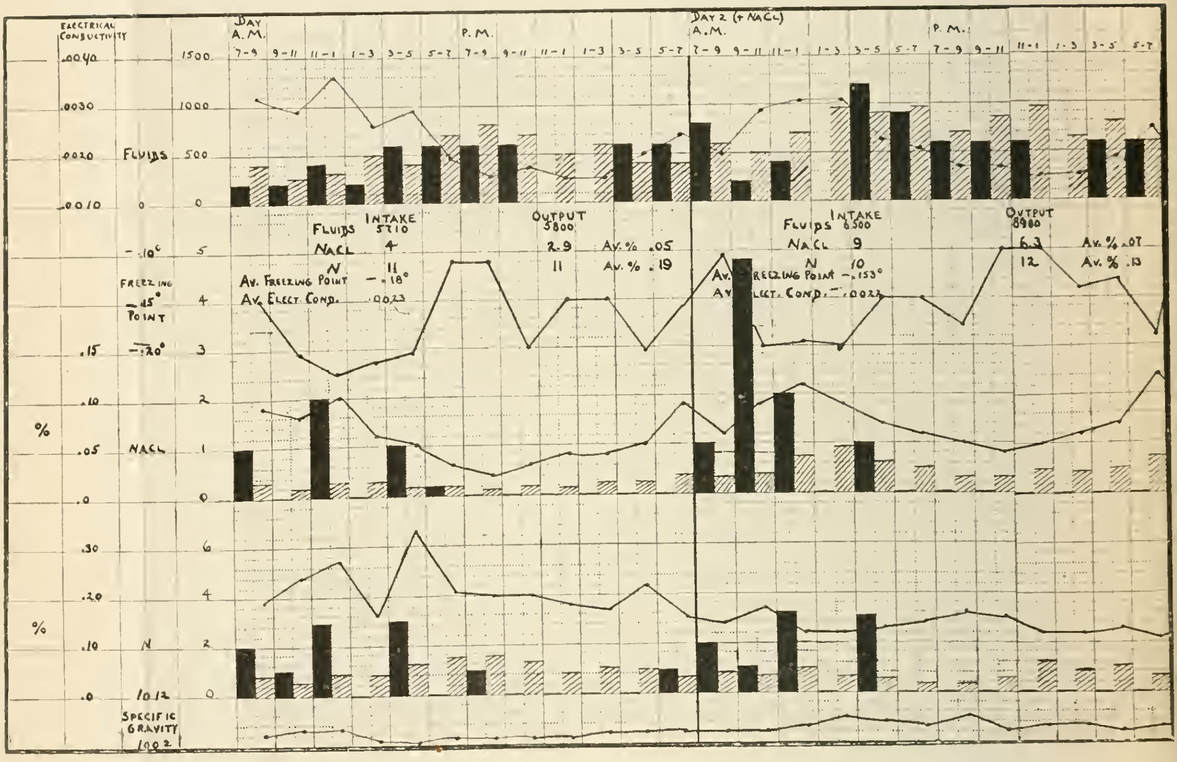

Chart 2.—The effect of sodium chlorid, urea, and other diuretics on two-hourly specimens of urine.
by Kohlrausch's method. The electrical resistance was measured in reciprocal ohms per centimeter cube
Messungen. Englemann, Leipsic, Ed. 3, 1910, p. 473). It gives the author great pleasure to thank Dr.

When 25 gm. of sodium chlorid were given, there was slight increase in its concentration in the urine for the following forty-eight hours, which would suggest that the tubules were still functionally active. This was confirmed by a normal excretion time for potassium iodid. The chlorid, however, had a striking effect on the renal vascular system. For three days following its administration there was a

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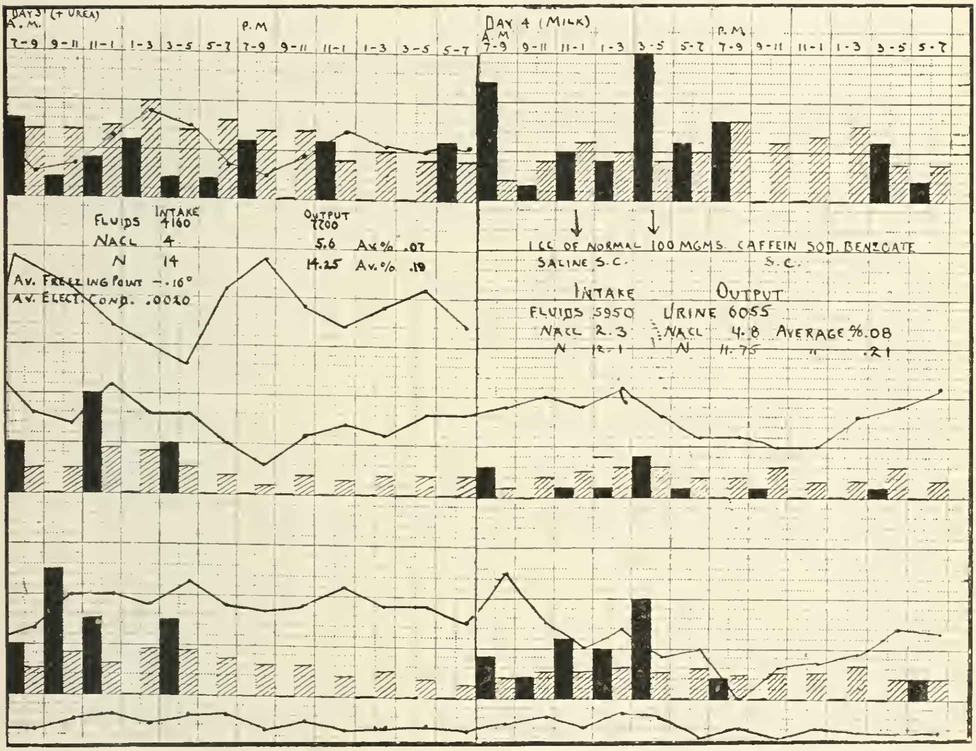
28. Benczur: Wien. klin. Wchnschr., 1910, xxiv, 890.
29. Geyelin, W. Rawle: A Clinical Study of Amylase in the Urine, The Archives Int. Med., 1914, xiii, 96.
30. Strauss: Deutsch. med. Wchnschr., 1912, iv, 163.
31. Rosenthal: Berl. klin. Wchnschr., 1912, xlix, 1265.

polyuria, so that more than 3 liters over the fluid intake was excreted. Lactose came through normally, as might be expected, since Schlayer has already shown that excessive amounts of urine may increase its elimination time. Thus the case showed the typical findings of "vascular hyposthenuria" with hypersensitive vessels.

Since large amounts of sodium chlorid appeared to have such a marked effect on the total amount of urine excreted, it was important to discover the rate of elimination of water and salt; whether smaller amounts of this drug might have the same effect according to Stuber's[32] observations that certain cases of diabetes insipidus reacted to very minute doses, and whether other diuretic substances acted as irritants to the kidney. Accordingly, a four-day experiment was undertaken in which specimens of urine were analyzed at two-hour intervals for nitrogen and sodium chlorid, and in which the freezing-point and electrical conductivity were determined. The first day served as control; on the second day 5 gm. of sodium chlorid was added to the diet; on



The freezing-point was determined by Beckmann's method, and the electrical conductivity of urine according to Oswald-Luther's tables. (Oswald-Luther: Physiko-Chemische Paul Dudley Lamson for his kindness in making these readings.

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32. Stuber: Deutsch. Arch. f. klin. Med., civ, 394.

the third day 10 gm. of urea; on the fourth day the diet was changed to milk, and in the morning 1 c.c. of sterile saline solution was injected subcutaneously, while in the afternoon 100 mg. of caffein sodium benzoate. The results of these observations are shown in Chart 2.

It became evident from the total amount of urine excreted that both sodium chlorid and urea acted in producing a polyuria, that no reaction was obtained from the minute amount of sodium chlorid contained in 1 c.c. of saline solution, and that caffein sodium benzoate possibly had a diuretic action as more fluid was excreted in the hours following its injection than before. From the analysis of specimens from hour to hour it appeared that the rate of fluid excretion under normal conditions was relatively constant and did not bear a definite relation to the amount of fluid ingested. The rate of chlorid and nitrogen excretion, also, was approximately level. When the sodium chlorid and nitrogen were added a diuretic action was obtained during the entire following twenty-four hours, but was most pronounced in the early hours of the day when it might be assumed that the concentration of these substances in the blood was highest. It is interesting to note that the total molecular concentration of the urine, as estimated by the freezing-point, was less on the day that sodium chlorid was added than on either the day preceding or that following. The electrical conductivity was nearly constant, but showed changes from hour to hour, and as might be expected averaged slightly lower on the days when the larger amount of sodium chlorid was excreted than on the control day. Meyer considers that such a reaction affords evidence of an inability of the kidney to increase its total concentrative powers. But as already pointed out by Meyer and Forschbach, the concentration per hundred c.c. of urine showed definite variations from hour to hour; so here again, according to Schlayer's views, is evidence of vascular hyposthenuria with hypersensitive vessels. The concentration of the urine varied, suggesting that the concentrative faculties of the kidney were still present, yet chlorid and urea acted, in proportion to their concentration in the blood, as diuretics on the irritable renal vessels. In other words, the sodium chlorid and urea acted on the kidney in much the same fashion as do intravenous injections of these diuretics in animals. Diuresis was most pronounced at first, and gradually diminished in ratio to a diminishing concentration in the blood.

Meyer[18] had already commented on the reaction of certain cases of diabetes insipidus to small doses of theocin. He found that this drug did not affect the total amount of fluid excreted, but increased the concentration of sodium chlorid in the urine so that during the twenty-four hours following its administration the excretion of salt was increased. When theocin was omitted, the chlorid output diminished until the normal balance was regained. In this connection Mosenthal and Schlayer[33] have conducted interesting experiments in relation to renal fatigue by diuretics. They produced uranium or chromate nephritis in rabbits and studied the effect of repeated injections of sodium chlorid or caffein on the total output of water and salt, and on the volume of the kidney as recorded in an oncometer. In the early stage of chromate nephritis sodium chlorid produced an increased outflow of urine and salt with dilatation of the renal vessels, while subsequent injections of caffein caused a diminishing output of these substances with vascular contraction. In uranium nephritis, on the other hand, directly opposite results were obtained. Injections of sodium chlorid alone were ineffective in causing diuresis, while caffein, given later, reestablished the flow of urine. Mosenthal and Schlayer explained these findings on the assumption that in one type of lesion the kidney was fatigued by caffein with abnormal rapidity, and in the other type of lesion by sodium chlorid.

From the results of these studies it might be imagined that certain cases of diabetes insipidus with vessels "hypersensitive" to chlorid stimulation might be fatigued by the superposition of purin or other diuretics. In consequence, it might be expected that such diuretics would not increase the output of fluid, but might rather actually diminish it, and that the concentration of chlorid and its total output might be normal or even increased providing the tubules were capable of functional activity. The following experiments were made to test this hypothesis. On one day the patient was given 5 gm. of sodium chlorid; on another 0.6 gm. of theocin in divided doses; on a third, 5 gm. of sodium chlorid and 0.75 gm. of theocin, and on a fourth day 0.9 gm. of theobromin sodium salicylate (diuretin) in divided doses. The results of this treatment are shown in Chart 3.

It is seen that theocin and theobromin sodium salicylate alone had no diuretic action, nor did they increase the total output of sodium chlorid. In the presence of additional chlorid, theocin had no effect in preventing diuresis, but on the other hand it did not increase the difference between the intake and output of fluid and therefore seemed to have no independent action. It is interesting that when theocin and salt were given together, a larger total output of chlorid was obtained than when no theocin was included. The concentration was not increased, however, so no definite statement as to theocin effect can be made. Rather than to consider that theocin increased the output of salt, it is easier to believe the combination of drugs made the patient more thirsty than chlorid alone, and that a certain amount of excess salt was washed through the kidney in the large fluid bulk. These observations show definitely that, in our case at least, theocin superimposed on chlorid did not fatigue the hypersensitive renal vessels.

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[33] Mosenthal and Schlayer: Deutsch. Arch. f. klin. Med., 1913, cxi, 217.

On the whole, these various tests for kidney function brought out several interesting features. According to Schlayer's views, the patient reacted to the differential tests for renal function as do cases of nephritis with "hypersensitive" vessels, but with normal renal function in other respects. There was a constant "vascular" hyposthenuria; "tubular" function was normal according to the iodid test and because the concentrative power of the kidney was not entirely lost. The excretion time of lactose was normal, as was to be expected on account

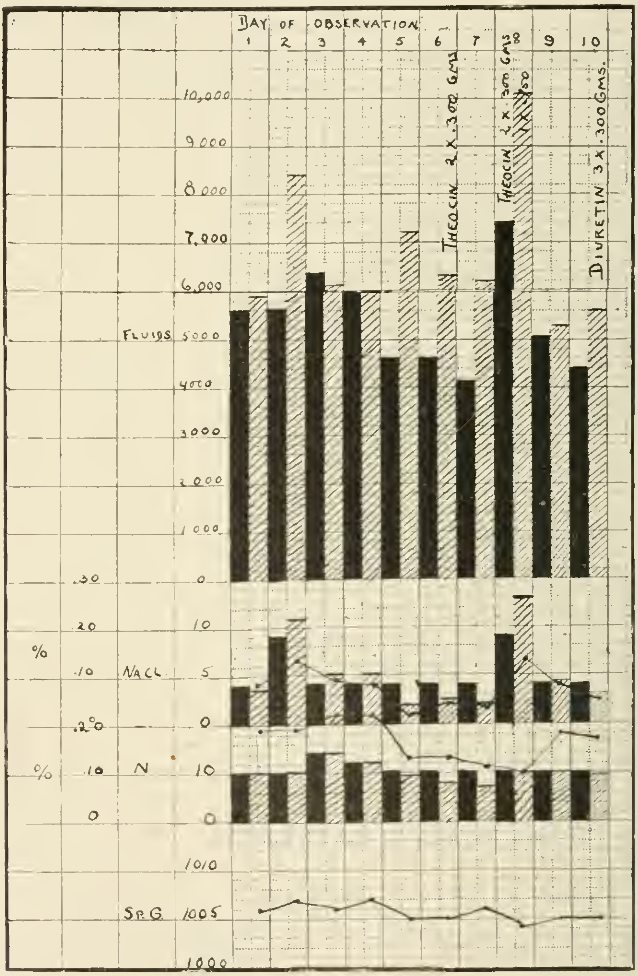

Chart 3.—The effect of diuretics on urinary output.

of the polyuria. These findings, however, justify the belief that no severe anatomical lesions existed in the kidney. This was confirmed by negative physical and urinary examinations, by a normal phenolsulphonephthalein elimination, and by a lack of rest-nitrogen accumulation in the blood.

There is insufficient evidence on which to draw any conclusions as to the underlying cause of the disease in this patient. Certainly, the renal findings are as well explained on the assumption of a lesion in a brain area without focal symptoms, which causes a hypersensitiveness of the renal vessels either by stimulation of the vasomotor nerves of the kidney or through hypersecretion of hypophyseal extract, as on the basis of a primary kidney disturbance. Either of these factors could produce a polyuria of the type encountered in which the renal vessels were hyperirritable to stimulation and in which the renal function was normal in other respects.

The treatment of diabetes insipidus has always been unsatisfactory. Certain cases of syphilitic origin have been reported which have shown improvement under the administration of iodids and mercury, but at least as many have been treated without benefit. Valerian has been advocated by the French school under Trosseau, but results from its use have been inconclusive. A gradual restriction of fluid intake has been suggested by Geigel,[34] Strubell,[35] Schwenkenbecher[22] and others. But this means has proved too uncomfortable for the patients to warrant its general usage. To illustrate the suffering of those patients whose fluids are limited, Trousseau[36] quotes the following case of a young man who was passing daily as much as 32 liters of urine.

> In the wards in which he was placed, he was subjected to an almost total abstinence from drink; and to quench his craving thirst he was given some ice or lemon to suck, allowing him to take food in accordance with the promptings of his appetite. The unfortunate young man resigned himself to this severe regimen, which he endured for eight months, although he suffered so cruelly from it that one day he seized the chamber-pot and drank the contents to the last drop.

Strubell's case is equally dramatic. The patient under observation was locked in a room with two iron-barred windows. Strubell kept the key. The patient, however, became so thirsty when his drink was cut down that once he captured a half liter of rain-water in a most complicated fashion, as it ran off the roof; once he drank the water in which he bathed, and once, 1,400 c.c. of his own urine. It is only fair to add that the recent work of Socin tends to show that a thirst cure may be borne without ill effect or too great discomfort.

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[34] Geigel: Deutsch. Arch. f. klin. Med., 1885. xxxvii, 51.

[35] Strubell: Deutsch. Arch. f. klin. Med., 1899, lxii, 89.

[36] Trousseau: Lectures on Clinical Medicine, New Sydenham Society. London, 1870, iii, 530.

A more rational form of therapy is to treat the patient not only for syphilis if it exists, but also according to the excretory capacity of his kidney, using a bland and sufficiently nourishing diet which will not excite a violent polyuria. Intercurrent infection has been observed by many writers to have a beneficial effect on diabetes insipidus. Külz[8] and Pauli[37] have emphasized this feature in the disease, and Strubell[35] has tested it experimentally by the production of an artificial fever from the subcutaneous injection of deutero-albumose. Finally, Herrick[38] has reported a case in which lumbar puncture seemed to relieve the symptoms strikingly. In our case, a month's antisyphilitic treatment has had no effect. The patient received four injections of salvarsan, and has been given mercury and iodid. At present his symptoms are unchanged, and the Wassermann reaction has not

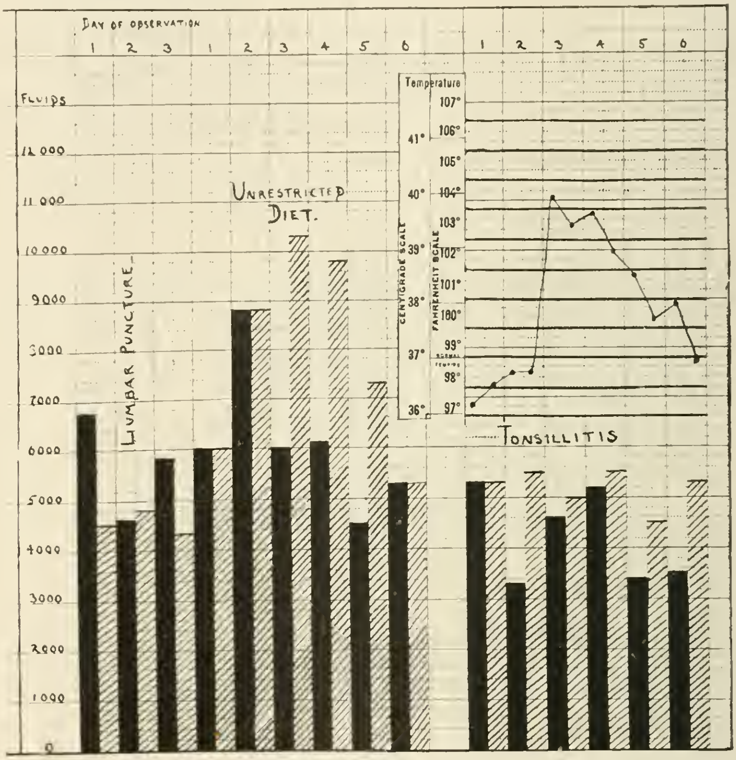

Chart 4.—The effect of lumbar puncture, of diet and of intercurrent fever on the output of urine.

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37. Pauli, Inaug. Diss., Zürich, 1891.

38. Herrick, James B.: Report of a Case of Diabetes Insipidus with Marked Reduction in the Amount of Urine Following Lumbar Puncture, The Archives Int. Med., 1912, x, 1.

diminished, although it is impossible to predict the eventual result of this form of treatment. The best results, therapeutically, have been obtained by giving the patient food which contained relatively small amounts of sodium chlorid and nitrogen. He was kept on a restricted diet for a considerable period, and, at last, as a reward, was allowed to eat anything he chose for three days. The results of this change of diet were so striking that the patient himself noticed them and asked to be given his former diet. From this it is evident that dietetic treatment may afford considerable relief to the symptoms. That acute infections and fever do not always benefit the disease was shown in this case. The patient developed an acute tonsillitis with fever for three and one-half days, during and immediately following which there was no definite change in the amount of urine excreted. Lumbar puncture, also, afforded no relief to the patient's symptoms. These facts are illustrated in graphic form by Chart 4.

SUMMARY AND CONCLUSIONS

Frank's original definition for diabetes insipidus still holds, "a long-continued abnormally increased secretion of non-saccharine urine which is not caused by a diseased condition of the kidneys." There are three much-discussed theories in regard to the pathologic physiology of the disease: (1) that it is due to faulty concentrative powers on the part of the kidney; (2) that it is a primary polydipsia with normal renal function; (3) that it is a symptomatic polyuria induced by stimulation of the kidney from a variety of causes, but in a certain number from hypersecretion of the hypophysis. It is a chronic disease not amenable to treatment. Since it is so frequently of syphilitic origin, syphilis should be treated when it is present. In other respects, each case must be treated individually, in accordance with the excretory capacity of the kidney, and with avoidance of substances which exaggerate the polyuria.

Tests for renal function in the case reported showed that the kidney as a whole was normal, but gave findings characteristic for vascular hyposthenuria with vessels hypersensitive to chlorid stimulation. Other diuretic substances and minute amounts of salt were inactive. The concentrative powers of the kidney were not entirely lost. This case therefore is probably not due to a primary lack of ability to concentrate on the part of the kidney. Variations in diet produced marked variations in both subjective and objective symptoms. Lumbar puncture and intercurrent fever had no visible effect on the disease, nor did one month's antiluetic treatment.

CONTRIBUTIONS TO THE EXPERIMENTAL PATHOLOGY OF THE STOMACH

I. EXPERIMENTAL PYLORIC STENOSIS *

W. W. HAMBURGER, M.D., AND J. C. FRIEDMAN, M.D.
CHICAGO

The sequence of events responsible for the clinical and pathological findings in disease of the stomach is often difficult if not impossible to analyze. The difficulty is directly proportional to the number and complexity of the processes involved. For example, in chronic gastric ulcer one attempts to analyze and properly coordinate the increase or decrease of gastric acidity, secretion, and motility; pyloric spasm or stenosis; dilated or hypertrophied stomach, etc. Through long clinical custom certain views are current as to the cause and effect of these interrelating processes, without much question as to the validity of these views or without subjecting them to experimental proof.

It has seemed to us that in view of the complexity of the problem of chronic ulcer of the stomach, these several processes should be separated and studied individually, a method, as Bolton[1] points out, which has not hitherto been adopted.

The following paper is presented as the first of a series of reports concerning the experimental pathology of the stomach and contains the results of producing primary partial stenosis of the pylorus in a series of twenty-nine dogs. It includes incidental reference to the relation of pyloric obstruction to chronic ulcer, a question which will be discussed at greater length in a subsequent paper.

METHODS

Small male and female dogs weighing from 6 to 8 kg. were used exclusively. Under usual surgical asepsis and complete surgical anesthesia an upper abdominal incision is made somewhat to the right of the mid line to insure easy access to the pyloric region. The stomach and antrum are pulled up into the wound and a fairly heavy silk ligature passed under the pylorus with the aid of an aneurysm needle and tied as near as possible to the duodenopyloric junction, avoiding the compression of large blood-vessels. The ligature is tied suffi-

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* Submitted for publication June 4, 1914.

* From the Morris Institute for Medical Research.

[1] Bolton: Proc. Roy. Soc. London, 1909-10, lxxxii, B 241.

ciently tight to produce permanent obstruction as evidenced by deep peristaltic waves in the antrum, cramp of the pylorus with temporary anemia, antiperistaltic waves, etc. Considerable experience is necessary to produce proper tension of the ligature, as in some instances it is tied too loosely, producing little, if any, obstruction, or too tightly, causing complete stenosis with death of the animal within from two to five days. The high mortality in many of our earlier dogs is to be attributed to this latter cause.

After experimenting with various test-meals and methods of introducing and withdrawing them, the following method was chosen as a routine: The dogs were fasted from eighteen to twenty hours over night and in the morning were given 100 gm. chopped lean beef and 300 gm. milk introduced by stomach-tube. In from fifty to sixty minutes 1/20 grain apomorphin hydrochlorid is injected hypodermatically, resulting in two to three minutes in the emesis of the test-meal, which is collected in a large evaporating dish. In the obstructed animals there is present, in addition to the meat and milk residue, large rests of beef, cartilage, bones, potatoes, carrot, etc. of food eaten days before. The apomorphin method possesses advantages over other methods of withdrawal by tube in obtaining total residue of stomach. The meals were examined for total quantity, free and total acidity, the finer chemical and microscopic examination being dispensed with as unessential.

In many of the dogs a second laparotomy was made in intervals of from ten days to three weeks, when 1 or 2 c.c. of 5 per cent. silver nitrate was injected at various points (pylorus, fundus, lesser curvature) producing acute ulcers. In four dogs (Nos. 11, 30, 31, 13) the pyloric ligature and injections were made simultaneously, resulting in all instances in complete obstruction and death of the animal. Two dogs (Nos. 44, 52) were injected daily with 1/5 grain pilocarpin hydrochlorid and 1/50 grain eserin sulphate respectively, to produce recurring stenosis from pyloric spasm.

RESULTS

The results of pyloric occlusion may be grouped in three divisions, dependent on the degree of obstruction.

Group 1.—Moderate stenosis (ligature tied loosely) resulting in little change in gastric acidity, motility or size (ten dogs—Table 1).

Group 2.—Marked obstruction (ligature tied sufficiently tight to cause marked peristalsis and anemia) resulting in definite change in motility (decrease) acidity (increase) and size (dilatation and hypertrophy) (nine dogs—Table 3).

Group 3.—Complete obstruction (from occluding ligature or edema from silver nitrate injection) resulting in inanition, vomiting, convulsions and death of the animal in from forty-eight to one hundred and twenty hours (ten dogs—Table 4).

Group 1.—This is perhaps the most interesting of the series, demonstrating the resisting power of the dog's stomach, as indicated by maintenance of normal acidity, motility, and size in the presence of a pyloric ligature tied for obstruction and producing at time of operation definite pyloric obstruction. For the most part these animals showed no loss in weight or cachexia, ate their food greedily without apparent discomfort, grew fat and sleek in spite of their occluding ligatures; and had every evidence of good care and health in marked contrast to their appearance on entrance into the laboratory.

TABLE 1.—MODERATE –

Dog No.	Test Meal Before Operation			First Operation	Test Meal After First Operation		
	Date	Amount	Total Acid	Pyloric Ligature	Date	Amount	Total Acid
41	12/23	200	37	12/26	....	....	....
51	2/5	200	51	2/6	2/17	350	54
35	....	....	....	11/25	....	....	....
36	....	....	....	12/20	....	....	....
42	....	....	....	1/8	1/26	250	55
43	....	....	....	1/15	1/26	450	26
7	....	....	....	6/20	....	....	....
6	....	....	....	6/26	....	....	....
26	....	....	....	11/20	....	....	....
28	....	....	....	11/25	....	....	....

Test-Meals: We found for the most part a slight increase in total quantity and total acidity within from ten to fourteen days after pyloric obstruction. Two dogs (51, 42) following a primary hyperacidity gave a secondary drop to normal within six weeks of the original operation. Necropsy in one to three months after ligation revealed a slightly or moderately dilated stomach, the latter occurring in those dogs whose test-meals had shown hyperacidity. In Dog 41, however, in spite of an increase from 37 to 52 total acid in two months, the stomach was found of normal size.

The control test-meal findings in dogs in which no preliminary meal has been given, were obtained from a series of six normal dogs as shown in Table 2:

Most of the animals were necropsied in approximately two months after ligation. In every instance the silk ligature was found in place, buried usually in the serosa or muscularis coats and covered with connective tissue and adhesions, and adherent to the under surface of liver or gall-bladder.

Group 2.—In these animals, as a result of more securely tied ligature with marked pyloric obstruction, the changes of hyperacidity, hypersecretion, motor insufficiency, dilated and hypertrophied stomach were more marked. The dogs appeared ill at ease, grew thin and emaciated, refused food as if in pain, later became toxic and unable to stand, finally dying in from three weeks to two months from inanition and toxemia.

—Pyloric Stenosis

Second Operation	Test Meal After Second Operation			Necropsy			
$AgNO_3$	Date	Amt.	Total Acid	Date	Stomach	Ulcer	Scar
.....	2/13	425	25	2/28	Small	No ulcer	No scar
2/19	3/17	200	30	3/20	Small	No ulcer	No scar
12/18	1/26	150	38	2/20	Small slight hypertrophy	Two small healing ulcers	
12/30	1/26	350	26	2/20	Slightly dilated	Chronic pyloric ulcer	
.....	2/29	325	36	3/8	Slight dilatation	No ulcer	No scar
2/13	2/24	550	50	3/23	Slightly dilated; slight hypertrophy.	Chronic pyloric ulcer	
.....	.....	.....	.....	7/26	Fundus dilated; pyloric hypertrophy.		
.....	.....	.....	.....	7/10	Slight dilatation and hypertrophy		
12/13	.....	.....	.....	12/27	Small	Two acute ulcers	
12/10	.....	.....	.....	12/30	Slight dilatation	Both ulcers healing	

TABLE 2.—Test-Meal Findings in Six Normal Dogs

Dog No.	Total Quantity	Free Acid	Total Acid
1	150 c.c.	0	30
2	250 c.c.	0	31
3	350 c.c.	0	27
4	150 c.c.	0	25
5	200 c.c.	0	35
6	100 c.c.	0	32

Range—100-350 c.c.
Average—200 c.c.

Range—25-35
Average—30

Test-Meals: A marked increase in total quantity up to one liter of contents (five times normal) much of this being food residue but more supernatant fluid (hypersecretion). In all a marked primary hyperacidity was found, many showing later a secondary relative decrease (hypacidity) the total quantity remaining the same or

TABLE 3.—Marked -

Dog No.	Test Meal Before Operation			First Operation	Test Meal After First Operation		
	Date	Amount	Total Acid	Pyloric Ligature	Date	Amount	Total Acid
55	2/5	200	44	2/12	2/24	950	56
38	1/14	250	26	1/23	2/2	550	36
50	2/5	300	59	2/6	2/18	250	75
48	....	....	....	1/15	1/26	450	26
39	....	....	....	1/16	1/26	800	62
37	....	....	....	12/18	....	....	....
22	....	....	....	10/9	....	....	....
21	....	....	....	10/8	....	....	....
5	....	....	....	6/5	6/26	100	23

TABLE 4.—Complete—

Dog No.	Date	Operation	Postoperative Condition and Course
4	5/27	Pyloric ligature	Sudden death in 72 hours with convulsions
2	5/20	Pyloric ligature	Constant vomiting, gradual inanition
9	7/17	Pyloric ligature	Progressive inanition. Death in 96 hours
17	9/4	Pyloric ligature	Sudden death in 96 hours with convulsions
19	9/11	Pyloric ligature	Sudden death in 48 hours
24	11/27	Pyloric ligature and $AgNO_3$	Sudden death in 72 hours
13	8/22	Pyloric ligature	Sudden death in 96 hours
31	12/4	Pyloric ligature and $AgNO_3$	Sudden death in 96 hours
30	12/4	Pyloric ligature and $AgNO_3$	Sudden death in 48 hours
11	8/6	Pyloric ligature and $AgNO_3$	Sudden death in 72 hours

becoming higher. Number 48 showed a late hyperacidity of 50 without previous increase.

Group 3.—The animals in this series died more or less suddenly in from forty-eight to 120 hours. A few refused food, vomited what little water they drank and succumbed from progressive inanition and weakness. Most of the dogs were found dead in their cages in the morning, having been in good condition the previous evening, eating their food, standing and walking about the cage, etc. Two of them (Nos. 4 and 16) were seized suddenly with tonic convulsions resembling tetany and died within five minutes. The condition of these dogs suggests the clinical picture of gastric tetany seen occasion-

—Pyloric Stenosis

Second Operation	Test Meal After Second Operation			Necropsy		
$AgNO_3$	Date	Amt.	Total Acid	Date	Stomach	Ulcer
2/26	3/24	1050	42	4/13	Markedly dilated	Chronic pyloric ulcer
2/13	.....	.....	.....	2/26	Markedly dilated	No ulcer or scar
2/19	3/20	600	64	3/21	Markedly dilated and hypertrophied	Superficial pyloric ulcer
2/13	2/24	550	50	3/23	Slightly dilated—Marked hypertrophy	Two duodenal ulcers — two chronic pyloric ulcers
.....	2/24	525	62	2/28	Markedly dilated and hypertrophy	No ulcer or scar
12/27	1/26	350	50	2/20	Markedly dilated	Chronic pyloric ulcer
10/30	.....	.....	.....	11/20	Markedly dilated	Unhealed pyloric ulcer, healing lesser curv. ulcer
.....	.....	.....	....	10/30	Markedly dilated and hypertrophy	
7/17	.....	.....	.....	8/21	Markedly dilated	Chronic pyloric ulcer

—Pyloric Stenosis

Necropsy			
Date	Stomach	Pylorus	Stomach Contents
5/30	Slightly dilated	Complete obstruction	Moderate amount clear fluid
5/25	Markedly dilated	Edema of all coats	Glairy mucus covering fundus
7/21	Small	....	Small amount thick mucus
9/8	Small	Tightly contracted	50 c.c. thin milky fluid
9/13	Small	Strongly contracted	Mucoid fluid in stomach
11/30	Small	Thick and edematous	Four acute ulcers
8/26	Small	....	Small amount frothy mucus
12/6	Small	Edema of antrum	
8/9	Small	Firmly contracted	Little fluid in stomach

ally in benign pyloric obstruction and is in accord with the experimental findings of Kaufman,[2] MacCallum[3] and others.

At necropsy the pyloric antrum is tightly contracted as if in tonic spasm, the fundus slightly dilated, the stomach of usual size, containing a small amount of thin fluid and glairy mucus. In one case (No. 2) in which there had been constant vomiting, with progressive inanition and loss in weight, the animal living for five days, the stomach was quite markedly dilated.

DISCUSSION

The interpretation of the motor insufficiency, continuous secretion, hyperacidity, hypertrophy and dilatation of stomach produced by pyloric obstruction, we believe to be the following: As a result of the narrowed pyloric canal, food normally propelled into the duodenum within from six to twelve hours is retained twenty-four hours or even several days. During this time the pylorus is in a state of continuous peristaltic activity in attempts to drive the contents, through the obstruction, on into the intestines—a prolongation and intensification of the normal peristalsis described by Cannon.[4] The grinding and churning of the food caused by this increased peristalsis presses the gastric contents with increased violence against the pyloric mucosa, stimulating the production of a chemical stimulant by the gastric mucosa, which Edkins[5] has shown is responsible (after its absorption into the blood) for the continued secretion of gastric juice. We have found that as late as forty-four hours after test-meals, the obstructed dog's stomach contained large quantities of contents with high free (34) as well as total (90) acidity, a condition never seen in normal dogs. We conclude, therefore, that the hyperacidity resulting from obstruction is really a hypersecretion (continuous secretion) produced as described and persists as long as food is present in the stomach. The histology of hyperacidity will be reported on later.

The explanation of the hypertrophy and dilatation is not so clear, some dogs with marked obstruction having small stomachs with hypertrophied walls, others having marked dilatation, while dogs with only slight obstruction have very markedly dilated stomachs. In all probability the gastric muscle tonus plus a possible subacute or chronic gastritis are additional factors, although it may be entirely a muscle affair analogous to the response of the heart to a stenosis. Microscopic examination of the stomach wall is necessary to substantiate this. The results will be reserved for later publication.

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[2] Kaufmann: Am. Jour. Med. Sc., 1908, cxxvii, 606.

[3] MacCallum: Zentralbl. f. allg. Path., 1905, xvi, 385.

[4] Cannon: Mechanical Factors of Digestion, 1911, Chapter VI. Effect of Stomach Movements.

[5] Edkins: Jour. Physiol., 1906, xxxiv, 133.

In the dogs with *successful* partial pyloric obstruction (motor insufficiency, continuous secretion) the acute ulcers produced by silver nitrate injection were in many instances prevented from healing, or became chronic and progressive, particularly the pyloric ulcers (see Bolton). We believe that the constant mechanical trauma of the retained food, grinding forcibly back and forth on the raw surface, plus the continuous corrosive action of free hydrochloric acid (the stomach never being free from acid) are two of the most important factors in the production of chronic experimental ulcer.

CONCLUSIONS

1. Moderate pyloric stenosis in dogs (ligature around pylorus tied loosely) causes little or no change in stomach motility, acidity or size.

2. Marked obstruction (ligature tied sufficiently tight to produce marked peristalsis and anemia of the pylorus) causes motor insufficiency, continuous secretion (hyperacidity) and hypertrophy and dilatation of the stomach.

3. Complete occlusion (ligature or silver nitrate injection causing secondary occluding edema) results in inanition, vomiting, convulsions (gastric tetany) and death of the animal in from forty-eight to 120 hours.

4. The pyloric ligature (stenosis) results in a motor insufficiency with food retention which in turn sets up a continuous secretion with the constant presence of free hydrochloric acid.

5. Pyloric obstruction with resulting motor insufficiency and continuous secretion are probably the most important factors in the production of *chronic* experimental ulcer.

6. While the foregoing results concern *experimental* chronic ulcer, it is more than likely that primary partial pyloric stenosis (from whatever cause) with secondary motor insufficiency and continuous secretion are largely responsible for the progression and delayed healing of chronic ulcer in man.

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A CLINICAL AND BACTERIOLOGICAL STUDY OF HEXAMETHYLENAMIN AS A URINARY ANTISEPTIC *

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The increasing use of hexamethylenamin for pathological conditions has led to more systematic studies of its chemical and bactericidal properties, and, as a result, recent literature abounds with papers on the subject. Following the discovery by Nicolaier,[1] in 1894, that hexamethylenamin was broken down into formaldehyd in the urine, this drug was used extensively in infections of the genito-urinary tract. Up to 1902 the literature contained many papers on its use, a good review of which may be found in the paper by Lubowski.[2] For a few years after 1902 very little new work was done on hexamethylenamin as an antiseptic agent until 1908, when Crowe[3] demonstrated its presence in body-fluids other than the urine. Since that time there has been a revival of interest in this drug and its uses, and as a result it has become more or less a panacea for many ills to which the body is heir.

The findings of several of the recent workers not being entirely in harmony, and hexamethylenamin being used to a large extent in this hospital, it was deemed of interest to learn from personal experience in what conditions it is indicated. It was through the courtesy of the attending physicians of this hospital that permission was granted to use cases from their services and this opportunity is taken to thank them for their kindness and advice. Thanks are also due to the director of the laboratory for the use of laboratory records and material.

As Nicolaier[1] and others, Cammidge,[4] Citron,[5] Hanzlik and Collins,[6] Hinman,[8] Jordan,[8] Sollmann,[9] Talbot and Sisson,[10] Smith,[11] Vinde-

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* From the Mount Sinai Hospital, New York.

[1] Nicolaier: Centralbl. f. d. Med. Wissensch., 1894, xxxii, 897.

[2] Lubowski: Therapist, 1904, xiv, 114, 131, 151, 165.

[3] Crowe: Bull. Johns Hopkins Hosp., 1908, xix, 109.

[4] Cammidge: Lancet, London, 1901, ii, 174.

[5] Citron: Monatschr. u. d. Krankh. d Harn- u. Sexualapp., 1898, iii, 73.

[6] Hanzlik and Collins: Hexamethylenamin: The Liberation of Formaldehyd and the Antiseptic Efficiency Under Different Chemical and Biologic Conditions, The Archives Int. Med., 1913, xii, 578.

[7] Hinman: An Experimental Study of the Antiseptic Value in the Urine of the Internal Use of Hexamethylenamin, Jour. Am. Med. Assn., 1913, lxi, 1601.

[8] Jordan: Biochem. Jour., 1911, v, 279.

[9] Sollmann: Formaldehyd Derivatives, Jour. Am. Med. Assn., 1908, li, 818

[10] Talbot and Sisson: Boston. Med. and Surg. Jour., 1913, clxviii, 485.

[11] Smith: Boston Med. and Surg. Jour., 1913, clxviii, 713.

vogel,[12] have shown, the value of hexamethylenamin as a therapeutic agent depends on its decomposition into formaldehyd in acid mediums. That alkalies may also decompose hexamethylenamin into formaldehyd has been mentioned by few, Reché,[13] Jenness,[14] Sollmann.[9] This last worker, however, states that hexamethylenamin is only very slightly acted on by alkalies. From the nature of the chemical reaction this does not seem possible.

The reaction illustrates a true reversible equation as follows:

$$\underset{\text{Hexamethylenamin}}{(CH_2)\,6N_4} + \underset{\text{water}}{6H_2O} \rightleftarrows \underset{\text{Ammonia}}{4NH_3} + \underset{\text{formaldehyd}}{6CHO}$$

Inasmuch as this is a true reversible equation, the law of mass action would apply and the direction of the reaction would depend on the proportion of the reagents used. Hence, the least amount of acid which really acts as a hydrolyzing substance when added to hexamethylenamin will cause the reaction to go toward the formaldehyd end, and this has been shown very well by those who have used weak acids, such as 0.2 per cent. hydrochloric acid (Hanzlik and Collins[6]), dilute sodium acid phosphate and beta-oxybutyric acid (Smith[11]). In this present work 1 c.c. of a tenth-normal hydrochloric acid solution has been used and good tests for formaldehyd have been obtained. It has also been repeatedly shown that a neutral solution of hexamethylenamin itself, on being boiled or incubated, will break down into formaldehyd. This is undoubtedly due to the hydrolyzing action of the water. On the other hand, Hinman[7] and Hanzlik and Collins[6] have shown that alkalies added to hexamethylenamin prevent its decomposition. In testing out the bactericidal action of hexamethylenamin, as will be shown later, its decomposition could always be prevented by the addition of enough ammonium hydroxid to give it a concentration of 1 : 40,000. After incubating this solution, tests for formaldehyd were always negative. As a still further proof that formaldehyd is never present free in alkaline mediums after the administration of hexamethylenamin, may be mentioned the fact that it is never found in the alkaline fluids of the body. In the face of the foregoing facts it is difficult to explain the action of hexamethylenamin, or rather its derivative formaldehyd, in any but acid mediums.

There is, however, another phase to the question which must not be overlooked and which has not been dwelt on sufficiently. It has been known that both hexamethylenamin and formaldehyd combine with other substances found in the various fluids of the body. Nicolaier[15] mentions the fact that hexamethylenamin unites with uric acid to form a very soluble diformaldehyd uric acid. Citron[5] has stated that the bactericidal or inhibitory action is not due to formaldehyd, but to a sodium compound of it. Kobert[16] says that hexamethylenamin breaks down into ammonia and formaldehyd and forms with insoluble urates, soluble salts of formaldehyd uric acid. Hammarsten[17] and Cammidge[4] both state that when formaldehyd is added to urea a combination takes place. The reaction between formaldehyd and albumin is well known in the use of formaldehyd as a hardening substance. Schwarz[18] has shown that formaldehyd in combination with albumin forms methylene-albuminate combinations. Therefore, the question arises whether the favorable results obtained in some mediums, especially alkaline mediums, are not due to some of these compounds of hexamethylenamin or formaldehyd which may be inhibitory or bactericidal. Such combinations would mask the tests for free formaldehyd. There is still the possibility that fluids which are normally alkaline, in pathological conditions may become just acid enough to liberate free formaldehyd, which immediately combines with the increased amount of albumin present forming a new compound in itself, also either inhibitory or bactericidal. The ammonia liberated from the hexamethylenamin at the same time that the formaldehyd is formed, would neutralize the acid present and the fluid would remain neutral or alkaline. This would be one theory to explain the beneficial action of hexamethylenamin in some cases of meningitis in which the organisms present are acid-producing organisms. It would be of interest to study the bactericidal actions of some of these compounds of hexamethylenamin or formaldehyd.

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[12] Vindevogel: Ann. soc. Roy. d. sc. med. Bruxelles, 1902, li, 20.

[13] Reché: Inaug. Dissert., Breslau, 1902.

[14] Jenness: Burnam's Test for Formaldehyd in the Urine, Jour. Am. Med. Assn., 1913, lx, 662.

There is hardly a secretion or fluid in the body in which hexamethylenamin after administration is not found. It can always be demonstrated in urines of those using the drug, in the saliva (Hanzlik,[19] Crowe,[3] Burnham[20]), in the middle ear and nasal sinuses (Barton,[21] Leibecke[22]), in the bronchi (Heitmueller,[23] Leibecke[22]); in the aqueous and vitreous humors of the eye (Whitman,[24] Hanzlik and Collins[6]), in

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[15] Nicolaier: Arch. f. klin. Med., 1904, lxxxi, 181.

[16] Kobert: Lehrbuch der Intoxicationen. 1906, ii, 1116.

[17] Hammarsten: Text-Book of Physiol. Chem., 1908, p. 556.

[18] Schwarz: Ztschr. f. physiol. Chem., 1900, xxxi, 36.

[19] Hanzlik: The Excretion of Hexamethylenamin in the Saliva. Jour. Am. Med. Assn., 1910, liv, 1940.

[20] Burnham: An Experimental Investigation of the Value of Hexamethylenamin and Allied Compounds, The Archives Int. Med., 1912, x, 324.

[21] Barton: Bost. Med. and Surg. Jour., 1910, clxii, 896.

[22] Liebecke: Berl. klin. Wchnschr., 1913, l, 1698.

[23] Heitmueller: Excretion of Hexamethylenamin, Jour. Am. Med. Assn., 1910, liv, 1223.

[24] Whitman: Jour. Ophth., 1912, xli, 604.

tears (Gradle[25]); in synovial fluids (Crowe,[3] Hanzlik and Collins[6]); in pleural fluids (Hanzlik and Collins,[6] Shattuck[26]); in pericardial fluids (Hanzlik and Collins[6]); in peritoneal fluids (Leibecke[22]); in pancreatic juice (Crowe[3]); in bile (Crowe,[3] Burnham[20]); in milk (Leibecke,[22] Crowe,[3] Hanzlik and Collins,[6] Rieder,[27] Buchura,[28] Schmidt and Schroter[29]); in eruptions on the body (Sachs[30]); in lochia (Hanzlik and Collins[6]); in cerebrospinal fluids (Burnham,[20] Crowe,[3] Flexner and Clarke,[31] Hald,[32] Hanzlik and Collins,[6] Ibraham,[33] Leibecke[22]), and in blood-serum (Hald,[32] Vindevogel[12]).

In this hospital some indications for the use of hexamethylenamin have been:

1. As an antiseptic in infections of the genito-urinary tract such as pyelitis, cystitis, pyonephrosis and infections following calculi.
2. As a prophylactic preceding catheterizations.
3. As a urinary antiseptic in typhoid fever.

In this work the investigations have been confined to the examination of urines in those cases mentioned above. The urines in all cases have been examined for the presence of hexamethylenamin, formaldehyd, and for the amount of acidity. The use of the Rimini[34] test by Burnham[20] afforded an easy method for the recognition of formaldehyd and at the same time the results obtained could be used in comparison with those of others. The test consists of the use of three solutions:

1. Phenylhydrazin hydrochlorid, a 0.5 per cent. aqueous solution, 3 drops.
2. Sodium nitroprussid, a 5 per cent. solution, 3 drops.
3. Sodium hydroxid, saturated solution, 2 or 3 drops.

These solutions are added to from 5 to 10 c.c. of urine. In comparing the colors obtained with solutions of formaldehyd of known concentrations the results agree with Smith,[11] who found that in solutions of 1:10,000 or stronger, a blue color is obtained, 1:15,000 to 1:25,000 a greenish black, and weaker solutions, an intense green.

The acidity was determined by litmus paper and by titrating 10 c.c. of urine with tenth-normal sodium hydroxid solution using phenol-

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25. Gradle: Ophth. Rec., 1911, xx, 110.
26. Shattuck: Boston Med. and Surg. Jour., 1911, clxiv, 842.
27. Rieder: Monatschr. f. Kinderheilk., 1912, xi, 80.
28. Bucura: Ztschr. f. exper. Path. u. Therap., 1907, iv, 398.
29. Schmidt and Schroter: Zentralbl. f. d. Physiol. u. Path. d. Stoffwechsel., v, 129.
30. Sachs: Wien. klin. Wchnschr., 1912, xxv, 153.
31. Flexner and Clark: Experimental Poliomyelitis in Monkeys, Jour. Am. Med. Assn., 1911, lvi, 585.
32. Hald: Arch. f. exper. Path. u. Pharmakol., 1911, lxiv, 329.
33. Ibraham: Med. Klin., 1910, xxxvi, 1893.
34. Rimini: Ann. di Farmacol., 1897, xvii, 97.

phthalein as an indicator. The litmus paper did not always agree with the acidity shown to phenolphthalein, especially when the titration required from 0.3 to 0.6 c.c. of tenth-normal sodium hydroxid solution. In these cases the litmus was either neutral or faintly alkaline. The acidity of the urines expressed in terms of 100 c.c. of urine ranged from 3 to 144. It was found that the acidity of the urines obtained within eight hours following an operation was usually high, from 50 to 80, one, 195, a few as low as from 30 to 40. It was also found that catheterized urines from patients not operated on ranged between 60 and 70. The average urine required from 3 to 3.5 c.c. of tenth-normal sodium hydroxid to neutralize 10 c.c. of urine. This agrees with Hinman,[7] who found the average to be 3.1 c.c.

The urines from 91 patients were examined. The greater number of these patients received 7 gm. of hexamethylenamin three times a day. Most of the urines were examined twice and some of them as many as six times. About 250 urines were examined. The 91 cases may be divided as follows: surgical 42, medical 32, genito-urinary 17. The urines of all of the 91 patients gave a test for formaldehyd varying from 1 : 20,000 to 1 : 5,000. The urines from 72 patients showed the presence of formaldehyd in the strength of 1 : 10,000. These results agree with those of Smith,[11] Talbot and Sisson,[10] Hanzlik and Collins,[6] Hinman,[7] all of whom found formaldehyd in over 97 per cent. of acid urines in patients using hexamethylenamin. Burnham,[20] L'Esperance,[35] Jenness[14] found formaldehyd in about 50 per cent. of their cases. In explanation of this fact Burnham proposed the theory that there is a selective action on the part of renal cells toward the breaking down of hexamethylenamin. Hinman[7] has disproved this by catheterizing the ureters of 23 patients, testing these urines and also the urines obtained at the same time from the bladders. Only 5 ureteral urines showed formaldehyd. Of the bladder urines only 3 failed to show formaldehyd. Somewhat similar results were found in 3 of the 91 cases reported in this paper. In these the ureteral urines were negative for formaldehyd, whereas the urines from the bladder showed its presence. In those cases in which formaldehyd is liberated in the kidneys it is probably due to the higher than normal acidity of the urine in the kidneys. These are undoubtedly the cases that develop a hematuria or albuminuria. It may be concluded, therefore, that in almost all cases hexamethylenamin is excreted into the bladder unaltered and then, under the influence of the acid in the urine and the warmth of the body, is broken down into formaldehyd.

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[35] L'Esperance: Bost. Med. and Surg. Jour., 1912, clxvii, 577.

The laboratory records of 100 urines, catheterized under aseptic conditions, show that the following organisms had been isolated:

Bacillus coli	64
B. pyocyaneus	11
B. proteus	4
B. paratyphosus	1
B. typhosus	1
B. mucosus capsulatus group	2
Staphylococcus aureus	8
S. citreus	1
S. pyogenes	6
B. fecalis alkaligenes	2
Total	100

These were recovered in the following cases:

Pyelitis	59
Pyonephrosis	12
Neprolithiasis	7
Cystitis	10
Miscellaneous	12

In this series of cases the colon bacillus was the most common invader of the urinary tract, occurring in 64 per cent. of the cases. To be of real therapeutic value, the hexamethylenamin or its derivative would have to be excreted in a concentration strong enough to be either inhibitory or bactericidal. Considerable work has been done on the antiseptic value of hexamethylenamin and formaldehyd. Stern[36] mentions patients with cystitis and pyelitis who had received hexamethylenmain for a long time and from whose urines he had isolated either the *Bacillus coli*, the *B. lactis aerogenes*, *B. pyocyaneus*, or the *Staphylococcus aureus*. Richardson[37] was one of the first to show that the *B. typhosus* disappeared very quickly from the urine of typhoid patients. Horton-Smith[38] and Biss[39] also found this to be true.

Experiments have been carried on in which the urines of patients who had received large doses of hexamethylenamin were inoculated with different organisms. Forcart[40] inoculated these urines with *B. coli*, *B. typhosus*, *B. proteus*, *B. pyocyaneus*, streptococcus, *Staphylococcus albus* and *S. aureus*. These were incubated for five days. He found that the colon bacillus was the most resistant.

Others have tested the inhibitory or bactericidal power of solutions of hexamethylenamin. The solutions have been added to cultures or emulsions of bacteria and incubated, or the solutions have been inoculated and incubated. It must be borne in mind that hexamethylenamin by itself when incubated or warmed gently will liberate a small amount

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36. Stern: München. med. Wchnschr., 1910, lvii, 2273.
37. Richardson: Jour. Exper. Med., 1899, iv, 14.
38. Horton-Smith: Brit. Med. Jour., 1900, Pt. 1, 827.
39. Biss: Edinburgh Med. Jour., 1902, New Series, xii, 337.
40. Forcart: Med. Klin., 1908, iv, 335.

of formaldehyd and in those experiments in which weaker concentrations of hexamethylenamin were inhibitory or bactericidal it may have been this small amount of formaldehyd that produced the effect. The decomposition of hexamethylenamin, as has already been stated, may be prevented by the smallest amount of alkali.

Keuthe[41] states that 1 : 10,000 solution of hexamethylenamin inhibits the growth of *B. typhosus* while it requires a 1 : 3,000 solution to inhibit the *B. coli.* No mention is made of the possible action of formaldehyd which may have been liberated. Muller[42] dissolved hexamethylenamin in bouillon in the concentration of 1 : 1,000, and inoculated this with *B. coli, B. typhosus, Staphylococcus aureus* or *B. pyocyaneus.* These were incubated and there was no inhibition of growth. Ascitic fluid which was inoculated with *B. coli* was also treated with a 1 per cent. solution of hexamethylenamin and incubated. There was no inhibition at the end of forty-eight hours. Goetzl and Salus[43] found a 5 per cent. solution bactericidal for *B. typhosus, B. coli, B. proteus* and staphylococci. Cammidge,[4] on the other hand, found that although a 7.5 per cent. solution killed completely the typhoid bacillus that the same solution only inhibited the growth of *B. coli* and *Staphylococcus aureus.* Results are different when an alkali is first added to a solution of hexamethylenamin to prevent its decomposition into formaldehyd and then this solution is inoculated and incubated. Burnham[20] inoculated 5 per cent. and 10 per cent. solutions of hexamethylenamin containing a slight amount of ammonium hydroxid with *B. coli, B. typhosus, B. pyocyaneus,* streptococcus and *Staphylococcus aureus.* These were incubated and all of the organisms survived. Hanzlik and Collins[6] inoculated agar mediums containing sodium carbonate or ammonium hydroxid and hexamethylenamin with bacteria and found that it was not bactericidal. Agar mediums containing hexamethylenamin and no alkali showed no growth when the concentration of the hexamethylenamin was 1 : 50 or 1 : 100. In weaker concentration there was growth.

The action of a 10 per cent. solution of hexamethylenamin was tried here on various organisms. To some of these solutions ammonium hydroxid was added so that there was a concentration of 1 : 40,000 of ammonium hydroxid. To others no ammonium hydroxid was added. For a control, sterile saline containing 1 : 40,000 ammonium hydroxid was used. All of the solutions were inoculated with equal amounts of a twenty-four-hour culture of one of the following bacteria: *B.*

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[41] Keuthe: Inaug. Diss., Heidelberg, 1902; quoted in Centralbl. f. Bakteriol., 1902, xxxii, 379.

[42] Muller: Deutsch. Aerzte. Ztschr., 1903, viii; quoted in Centralbl. f. Bakteriol., 1902, xxxiii, 808.

[43] Gotzl and Salus: Ztschr. f. klin. Med., 1902, xlv, 427.

typhosus, B. coli, B. paratyphosus, B. acidi lacti, B. lactus aerogenes, B. pyocyaneus, streptococcus and *Staphylococcus aureus.* These were incubated for twenty-four hours at 37 C. (98.6 F.) and a subculture taken. The results are given in Tables 1, 2 and 3.

TABLE 1.—Action of Ten Per Cent. Hexamethylenamin with No Ammonium Hydroxid on Growth of Organisms *

	Reaction	Formaldehyd	Bacteria
Staphylococcus aureus	N	1 : 20,000	Growth
Bacillus typhosus	V. F. A.	V. F.	Growth
B. coli	V. F. A.	V. F.	Growth
B. paratyphosus	N	1 : 10,000	No Growth
B. acidi lactis	N	1 : 10,000	No Growth
B. lactis aerogenes	N	None	Growth
B. pyocyaneus	N	V. F.	Growth
Streptococcus pyogenes	N	V. F.	Growth
Control Hexamethylenamin....	N	V. F.	—
Control Hexamethylenamin + NH_3	S. A.	V. F.	—

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* In this and the following tables, N = neutral; V. F. = very faint; F. V. A. = very faintly alkaline; S. A. = slightly alkaline.

It will be seen from the tables that incubating has effected a hydrolysis of those solutions of hexamethylenamin to which no ammonium hydroxid had been added. It may also be seen that the growth of only two of the organisms was inhibited by the small amount of formaldehyd liberated. This inhibition was present at the end of twenty-four hours. There was growth at the end of forty-eight hours. Similar experiments were carried on with bouillon containing 10 per cent. of hexamethylenamin and inoculated with *B. typhosus* or *B. paratyphosus.* Subcultures taken after twenty-four hours' incubation showed growth in all.

TABLE 2.—Action of Ten Per Cent. Hexamethylenamin, Containing 1 : 40,000 Ammonium Hydroxid on Growth of Organisms

	Reaction	Formaldehyd	Bacteria
Staphylococcus aureus	S. A.	0	Growth
Bacillus typhosus	S. A.	0	Growth
B. coli	S. A.	0	Growth
B. paratyphosus	S. A.	V. F.	Growth
B. acidi lactis	S. A.	V. F.	Growth
B. lactis aerogenes	S. A.	0	Growth
B. pyocyaneus	S. A.	0	Growth
Streptococcus pyogenes	S. A.	0	Growth

It may readily be seen that hexamethylenamin *per se* even up to a 10 per cent. solution has neither bactericidal nor marked inhibitory properties. Since hexamethylenamin is never present in the urine in concentrations as great as 10 per cent. it must be concluded that when an inhibitory or bactericidal action is shown in the urine it cannot be due to the hexamethylenamin.

TABLE 3.—Action of Saline Containing 1:40,000 Ammonium Hydroxid on Growth of Organisms

	Reaction	Bacteria
Staphylococcus aureus	S. A.	Growth
Bacillus typhosus	S. A.	Growth
B. coli	S. A.	Growth
B. paratyphosus	N	Growth
B. acidi lactis	N	Growth
B. lactis aerogenes	S. A.	Growth
B. pyocyaneus	S. A.	Growth
Streptococcus pyocyaneus	S. A.	Growth

From the work of Burnham,[20] Hess,[44] Kolle and Wassermann,[45] Kendall and Edwards,[46] Hanzlik and Collins,[6] Hinman,[7] Table 4 may be deduced.

TABLE 4.—Concentrations of Hexamethlenamin Required to Inhibit Growth and to Kill, Respectively, in Twenty-Four Hours

	To Inhibit	To Kill
Bacillus typhosus	1:15,000	1:5,000
B. coli	1: 7,000	1:1,000
B. pyocyaneus	1: 7,000	1:1,000
Streptococcus	1: 5,000	1:1,000
Staphylococcus aureus	1: 5,000	1:1,000

Formaldehyd to be of value must be liberated in strengths varying from 1:15,000 to 1:5,000, depending on the organism the growth of which is to be inhibited. To be of bactericidal value it must be liberated in strengths from 1:5,000 to 1:1,000, also depending on the organism. There is no simple chemical test for determining the exact amount of formaldehyd in urine. An approximate estimation may be made by diluting the urine with water in various proportions and testing these different proportions according to the Rimini method. By such a method formaldehyd was never demonstrated in a concentration greater than 1:5,000. Hence it is reasonable to assume that formaldehyd is probably never present in urine in solution strong enough to destroy any organism except the *B. typhosus*. It may, however, have an inhibitory action on other organisms.

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[44] Hess: Quoted by Borner, Centralbl. f. Bakteriol., Orig., 1910, liii, 415.

[45] Kolle and Wassermann: Handbuch. der pathogene Mikroorgamismen, ii, 387; iii, 115.

[46] Kendall and Edwards: Jour. Infect. Dis., 1911, viii, 250.

The acidity factor must also be considered. There is still some question as to whether or not there is a relationship existing between the amount of acid present and the amount of formaldehyd liberated. It has been shown by several that formaldehyd may be liberated by the smallest amount of free acid. Jordan[8] states that the percentage of formaldehyd, and with it the antiseptic power, increases with the acidity until the acidity has reached slightly above normal. He also states that when it reaches this point the urine remains sterile. To determine if there is any relation existing between the acidity, the amount of formaldehyd liberated and the resulting action of the two on bacteria the following experiments were conducted:

TABLE 5.—ACIDITY OF URINE, AMOUNT OF FORMALDEHYD LIBERATED AND RESULTING ACTION ON BACTERIA

	Time Examined	Acidity	Formaldehyd	Bacteria
Patient A—				
After Voiding				
Minutes	30	64	1 : 5,000	Sterile
Hours	24	51	1 : 5,000	Sterile
After Inoculation, hrs...	24	38	1 : 10,000	Growth
Patient B—				
After Voiding				
Minutes	30	68	1 : 5,000	Sterile
Hours	24	62	1 : 10,000	Sterile
After Inoculation, hrs...	24	62	1 : 10,000	Growth
Patient C—				
After Voiding				
Minutes	30	62	1 : 10,000	Sterile
Hours	24	60	1 : 10,000	Sterile
After Inoculation, hrs...	24	50	1 : 10,000	Sterile
Patient D—				
After Voiding				
Minutes	30	10	1 : 20,000	B. coli
Hours	24	Alkaline	1 : 25,000	
After Inoculation, hrs...				
Patient E—				
After Voiding				
Minutes	30	28	1 : 20,000	B. coli;
Hours	24	10	1 : 20,000	S. albus

Patients who had been given hexamethylenamin over a period of time were catheterized as aseptically as possible. The acidity and amount of formaldehyd was determined soon after voiding, and again twenty-four hours after the urine had been incubated. Cultures of these urines were taken and those which were found sterile were inoculated with cultures of *B. coli*. These were incubated again for twenty-four hours, and the acidity, amount of formaldehyd determined and subcultures taken. The results are given in Table 5.

In the two cases in which the acidity is low, that is, 10 and 28, the strength of formaldehyd found is less than in the urines of higher acidity. In these two cases the urines were collected through permanent catheters and the continuous flow probably accounts for the lower acidity. In three other urines in which the acidity was greater than normal the growth of the colon bacillus was only inhibited in one. The formaldehyd in this case had a concentration of 1 : 10,000. It may be concluded, therefore, that there is a relationship between the amount of acid present and the amount of formaldehyd found, and that in urines whose acidity is much higher than normal the combination of the increased acid and the formaldehyd may either inhibit or prevent the growth of bacteria other than the *B. typhosus*.

TABLE 6.—Effect of Increasing Acidity of Normal Sterile Urine After Twenty-Four Hours

	Acidity	Formaldehyd	Culture
1.—Normal urine	21		
2.—Normal urine, *B. coli*......	8	....	Growth
3.—10 c.c. normal urine, 7.5 per cent. hexamethylenamin..	8	1 : 20,000	
4.—10 c.c. normal urine, 7.5 per cent. hexamethylenamin, *B. coli*	8	1 : 20,000	Growth
5.—10 c.c. normal urine, acidity increased to 55, 7.5 per cent. hexamethylenamin, *B. coli*	20	1 : 10,000	Growth
6.—10 c.c. normal urine. Acidity increased to 80, 7.5 per cent. hexamethylenamin, *B. coli*	27	1 : 10,000	No Growth
7.—7.5 per cent. hexamethylenamin, *B. coli*............	Neutral	Negative	Growth

The effect of increasing the acidity was attempted in another way. Normal sterile urine of known acidity was used and the experiments shown in Table 6 tried. The acidity of this urine was 26. The amount of acidity was increased by adding tenth-normal hydrochloric acid.

Growth was inhibited in a urine with an initial acidity of 80 and a final formaldehyd strength of 1 : 10,000.

The acidity of urines of patients who had been using hexamethylenamin for some time was increased by the addition of a calculated amount of tenth-normal solution of hydrochloric acid. These were inoculated with the colon bacillus and incubated. The acidity in both cases was made up to 60.

In these two cases an acidity of 60 and a final formaldehyd strength of 1 : 10,000 was inhibitory. In these cases in which hydrochloric acid was added and growth was inhibited it must be borne in mind that the kind of acid used might also have been an important factor in causing the inhibition of growth. This is a reasonable assumption when we consider that a solution of formaldehyd of a concentration of 1 : 10,000 is not strong enough by itself to inhibit the growth of *B. coli.* It would, therefore, seem that the ideal method of treatment would be to increase the acidity of the urine to at least twice the normal by appropriate means at the same time that the hexamethylenamin is given. The only acid or acid salt that seems best adapted to this purpose is acid sodium phosphate. This, if pushed too hard, has its complications, the most common being diarrhea. It must also be borne in mind that the urinary tract is not a test-tube, and, whereas there are no dangers of destruction to tissue in a highly acid urine when the experiments are conducted in a test-tube, there may be considerable injury done when the experiment is carried on through the kidney. This is especially true when the formaldehyd is liberated in the kidney tubules or pelvis through the action of a highly acid urine. The large number of recorded cases of hematuria and albuminuria is a proof of this fact.

TABLE 7.—Effect of Increasing Acidity of Patients Who Had Used Hexamethylenamin for Some Time

	Time Examined	Acidity	Formaldehyd	Bacteria
Patient F.................				
After Voiding	Immediately	46	1 : 10.000	
	24 hours	44	1 : 10.000	
After Inoculation	24 hours	42	1 : 10,000	No growth
Patient G.................	Immediately	52	1 : 20.000	
After Voiding	24 hours	47	1 : 10.000	
After Inoculation	24 hours	40	1 : 10,000	No growth

SUMMARY

1. Hexamethylenamin, *per se,* in neutral solutions in concentrations up to 1 : 10 is neither inhibitory nor bactericidal.

2. Hexamethylenamin when given in doses of 7.5 grains three times a day is broken down into formaldehyd in all acid urines.

3. Formaldehyd is formed in the bladder in all cases except in those in which the acidity of the urine is higher than normal. In these cases some formaldehyd is formed in the kidneys.

4. The common invader of the urinary tract is the colon bacillus and the strength of formaldehyd necessary to inhibit its growth is a concentration of 1 : 5,000 or greater.

5. By testing different dilutions of urine it has been found that with a dose of 7 grains of hexamethylenamin three times a day, formaldehyd is never present in concentrations greater than 1 : 5,000.

6. The only organism which is destroyed or whose growth is inhibited by this concentration is the typhoid bacillus.

7. For the destruction or inhibition of growth of organisms other than the typhoid bacillus a high acidity is necessary. This high acidity in combination with the formaldehyd may produce injury to the kidney tissue.

8. Hexamethylenamin appears most efficacious as a prophylactic during the course of typhoid fever for the prevention of a pyelitis or cystitis. Its use in this connection has been demonstrated successfully both clinically and bacteriologically.

BLOOD-PLATELETS AND TUBERCULOSIS *

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So little is known concerning blood-platelets, and so little interest has been taken in them, that their description by Adami and Nichols[1] is almost fully quoted.

> If only because they play so important a part in the process of thrombosis it is necessary to have a clear understanding regarding the blood-platelets, or, more accurately, regarding what is known concerning their origin.
>
> Apart from this, since the advent of the Romanowsky stain and its modifications, they have of late years come in for increasing recognition.
>
> There is no longer any disposition to regard them as artefacts, but there is still dispute as to their exact significance and as to their unity or duality.
>
> They are small bodies of varying size, in general about 2μ in diameter, oval or pear-shaped, evidently labile, and varying in shape with slight compression by neighboring cells or platelets, tending to be present in smears in small groups (possibly as the result of rapid agglutination in shed blood).
>
> They are non-nucleated although containing often fine, central granules, which assume a redder tint with the Romanowsky stain in contrast to the bluer ground work.
>
> Pratt's careful studies show that they are present in the normal blood in greater numbers than the leukocytes, although the number shows wider variations from 200,000 to 700,000 per c.mm.
>
> Since their discovery and the early papers by Hayem, Bizzozero and Mrs. Ernest Hart, there have been very various views regarding their nature and mode of origin.
>
> 1. That they are precipitated globulin (Löwit. Woolbridge).
> 2. That they are products of disintegration of white corpuscles (Lilienfeld and Zenker).
> 3. That they are given off from disintegrating red corpuscles (Mono, Klebs and Arnold).
>
> The exquisite preparations made by J. H. Wright of Boston, demonstrate without possibility of doubt that some at least of the platelets, and those most typical, are normally derived from a particular order of cell, namely from the giant cells (megakaryocytes) of the bone-marrow.
>
> These cells give off processes projecting into the lumina of the capillaries, and it is the distal portions of these which become liberated into the blood as platelets. As such they may, as Schimmelbusch was the first to demonstrate, retain some power of ameboid movement, but the mode of their development sets at rest the debate as to the nature of the central staining granules, they in no sense represent a nucleus. . . .
>
> We must admit that in pernicious anemia the platelets are frequently (though not always) diminished in number, and that here there has been observed a lack of giant cells in the bone-marrow.

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* From the Laboratory of Cragmor Sanatorium, Colorado Springs, Colo.

[1] Adami and Nichols: Principles of Pathology, 1911.

In purpura they have at times been found completely absent. We know of no observations on the marrow giant cells in these cases. They are diminished also in typhoid, but increased in myelogenic leukemia and in pneumonia.

Port and Akijama[2] regard as normal 230,000 to 240,000 blood-platelets per cubic millimeter, with slight daily variations.

In acute infections (pneumonia, typhoid, scarlet fever) they found diminished numbers during the fever, but a large increase coincident with the drop in temperature. In chronic diseases they found very slight typical changes.

They think the blood-platelets play a part in the immunity processes as carriers of protective substances which are set free on their destruction.

Pagniez[3] doubts the necessity of platelets being present for blood coagulation, pointing out that lymph will coagulate without these elements. He reviews all the present knowledge of blood-platelets and suggests that some functions attributed to leukocytes may belong to the platelets, pointing out how difficult it is to procure leukocytes free from platelets.

Werbitzki[4] found that the blood of rabbits and horses contained antibodies for anthrax bacilli, which are different from serum bacteriolysin and the bactericidal substances of leukocytes, and which he traced to the platelets, but he found that the blood-platelets of the ox, hog, dog and man had no such substance. Werbitzki determined also that the blood-platelets of the above-mentioned animals and man had no bactericidal effect on typhoid bacilli, colon bacilli, staphylococci or the bacilli of dysentery or cholera.

Eminet[5] has demonstrated that blood-platelets in different infectious diseases possess different and specific affinities for stains.

He calls the blood-platelets *Soterozytes,* and shows in infection by the diphtheria bacillus, for instance, that these elements do not stain as they do in tuberculosis or scarlet fever cases, and vice versa. He considers therefore that the blood-platelets share in specific immune processes.

Grüber and Futaki[6] showed that the lymph or serum of a rabbit which has been heated to 65 C. (149 F.) can be given strong bactericidal power against anthrax bacilli if mixed with well-washed blood-pletelets (obtained by injecting sterile water under the skin of the rabbit).

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[2] Port and Akijama: Klinische Untersuchungen über Blutplattchen, Arch. f. klin. Med., cvi, Nos. 3 and 4.

[3] Pagniez: Arch. d. mal. du coeur, d. vaisseaux et due sang, January, 1909.

[4] Werbitzki: Ztschr. f. Hyg. u. Infections-krankh., 1911, lxviii, No. 1.

[5] Eminet, Charkow: Arch. f. Kinderh., lvii, Nos. 4 and 6.

[6] Gruber and Futaki: München. med. Wchnschr., 1907, No. 6.

Blood-plasma of rabbits and rats is completely without action against these bacilli. They believe the bactericidal power of the serum arises from a substance given to it by the platelets during the coagulation of the blood. This substance given up by the platelets is not thermolabile alexin, but really the same as is found in the serum of rabbits and rats. (Ottolenghi comes to another opinion.)

The platelet extract in physiological salt solution is not bactericidal, and the platelet extract in citrated blood-plasma is not active. (Ottolenghi says this is because the blood-platelets remain unchanged in this fluid and complement will only be freed when they undergo change as in the formation of fibrin or when they are mixed with serum or lymph.)

Blood-platelets were found to increase the bactericidal power of the normal serum of the rabbit, probably because the normal serum has many amboceptors, but not sufficient complement to activate all of them.

In studying the bactericidal power for anthrax bacilli of the leukocytes, Grüber and Futaki found those taken from "congestion lymph" more powerful than others.

The living plasma of the normal rabbit was found to be entirely free from antagonistic power to anthrax bacilli. The protective body gets into the serum only when the blood clots.

They conclude that the bactericidal substance furnished by the platelets to the serum is entirely different from the thermolabile alexin.

Ottolenghi[7] described the presence of complement in fibrin and found he could reactivate inactive serum and bring back the bactericidal power against certain bacteria by fibrin or its extract in salt solution.

Schneider[8] first demonstrated the method of procuring pure platelets by fractional centrifugation. He also demonstrated the connection between platelets and the formation of fibrin and blood-clotting.

Schneider also showed the possibilities of reactivating serum with an extract of platelets.

Aynaud[9] found that animals sensitized with platelets will react with red corpuscles and vice versa.

Aynaud[10] has noted the high amount of platelet content which is soluble in the solvent of fats, and also has noted the presence of phosphorus. He thinks these fats agree with the peculiar staining qualities of the platelets and their insolubility in the gastric juice, and their

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7. Ottolenghi: München. med Wchnschr., 1907, No. 17.
8. Schneider: München. med. Wchnschr., 1907, No. 3.
9. Aynaud: Compt. rend. Soc. de Biol., 1911, No. 37.
10. Aynaud: Presse méd., April 1, 1914.

digestion by the pancreatic juice lend argument in favor of a diffused nuclear substance in the element.

Wells[11] says: The blood-platelets are believed to be largely nucleoproteid, but little is known of their actual composition.

It is still undetermined just what part the platelets play in coagulation. Pratt, however, found that the number of platelets bore no relation to the coagulability of the blood, and that lymph which is free from platelets will coagulate.

Welch describes the steps in the formation of a thrombus after injury to the vessel-wall, as follows: First, there is an accumulation of blood-platelets adhering to the wall at the point of injury. Leukocytes which may be present in small numbers at the beginning, rapidly increase in number, collecting at the margins of the platelet masses and between them. Not until the leukocytes have accumulated does the fibrin appear.

TABLE 1.—Blood-Platelet Count of Dr. A. Taken at 12 Noon, at Colorado Srrings

Date	Platelets	Leukocytes
4/22/13	216,000	
4/23/13	184,000	
4/25/13	228,000	
4/26/13	184,000	
4/28/13	216,000	
4/29/13	288,000	6,000
4/30/13	222,000	6,000
5/ 1/13	180,000	6,200
5/ 2/13	228,000	6,800
5/ 3/13	168,000	5,800
5/ 5/13	228,000	6,000
5/ 6/13	220,000	6,000
5/ 8/13	222,000	6,000
5/12/13	164,000	6,000
5/14/13	248,000	6,000
5/15/13	208,000	5,400
5/17/13	244,000	5,800
5/20/13	180,000	6,200

The work to be presented consists of (1) a series of platelet counts in normal adults at sea-level and at an altitude of 6,000 feet; and a series in tuberculous patients and tuberculous animals at various stages, and (2) several series of experiments relative to the possible functions of blood-platelets.

The estimation of blood-platelets per cubic millimeter has until recently been most inexact on account of the lack of suitable technic. In the beginning of this work the metaphosphate method of Pratt was followed.

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[11] Wells: Chemical Pathology, 1907, p. 264.

All the counts below enumerated, however, were made according to the technic described by Wright and Kinnicutt.[12] Two parts of cresyl blue (1 : 300) are mixed with three parts of potassium cyanid (1 : 14,000) and rapidly filtered, then immediately used to dilute the blood 1 : 100. The blood must be from a free-flowing drop and the whole procedure must be carried out as quickly as possible.

The method appears reliable judging by the closeness of counts reported for the same individual.

The counts on Dr. A., a normal individual, given in Table 1, show the accuracy of the method which compares favorably with the accuracy of the successive enumerations of the white corpuscles.

Tables 2 and 3 give the counts of blood-platelets on two hundred normal male adults between the ages of 20 and 25, taken at sea-level (New York) and at an altitude of 6,000 feet (Colorado Springs). All the observations were made at 12 noon, when no digestion was occurring and no unusual exercise had been indulged in.

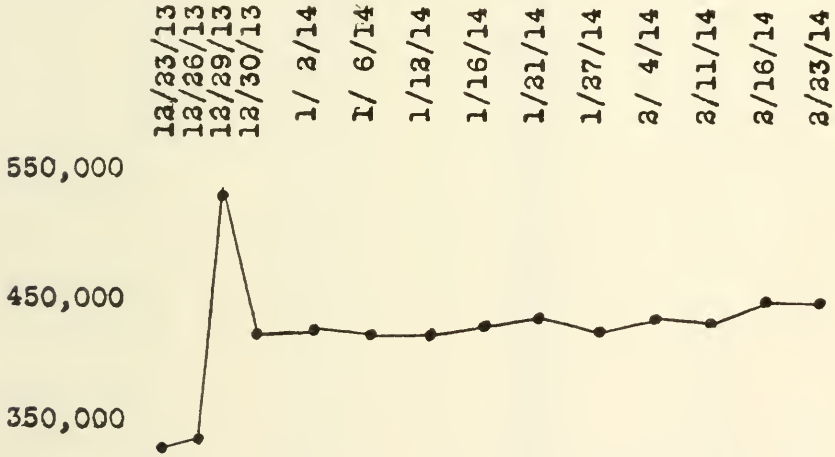

Chart showing composite of blood-platelets in five normal guinea-pigs for a period of two months.

The New York counts were made for us by Dr. Carolyn Rosenberg under the direction of Dr. T. W. Hastings of the Cornell Medical School. Dr. Gilbert and Dr. Rosenberg carefully went over the technic of counting together in New York.

These counts give an average of 302,000 blood-platelets at sea-level and 340,000 at an altitude of 6,000 feet. This is a percentage increase of 12.2.

The accompanying composite chart shows the average numbers of blood-platelets in five normal guinea-pigs immediately after arrival in

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[12] Wright, James Homer, and Kinnicutt, Roger: A New Method of Counting the Blood-Platelets for Clinical Purposes, The Journal A. M. A., May 20, 1911, p. 1457.

TABLE 2.—Blood-Platelet Count Made on Two Hundred Normal Male Adults in New York and Colorado Springs

No.	Cornell Students Platelet Count	Colorado College Students Platelet Count	No.
64	458,000	484,000	18
34	442,000	456,000	47
7	426,000	448,000	20
35	426,000	444,000	41
36	426,000	416,000	86
66	426,000	404,000	91
100	410,000	404,000	48
49	410,000	404,000	56
61	410,000	396,000	78
88	410,000	392,000	61
79	398,000	388,000	64
27	394,000	388,000	1
42	394,000	388,000	84
48	394,000	384,000	62
52	394,000	384,000	44
67	394,000	384,000	52
95	378,000	384,000	88
93	378,000	384,000	68
16	378,000	380,000	71
24	378,000	376,000	11
28	378,000	372,000	74
51	378,000	372,000	50
62	378,000	372,000	31
71	378,000	368,000	7
78	352,000	368,000	8
20	352,000	368,000	15
3	352,000	368,000	25
12	352,000	368,000	27
74	352,000	368,000	59
91	325,000	368,000	73
80	336,000	364,000	26
73	336,000	364,000	42
2	336,000	364,000	83
9	336,000	360,000	39
11	336,000	360,000	57
47	336,000	360,000	58
57	336,000	360,000	80
65	336,000	360,000	89
84	320,000	356,000	93
69	320,000	352,000	63
55	320,000	352,000	77
32	317,000	352,000	95
77	304,000	348,000	87
72	304,000	348,000	94
68	304,000	348,000	100
21	304,000	344,000	70
23	304,000	340,000	5
29	304,000	340,000	25
50	304,000	340,000	90
82	304,000	336,000	2
1	288,000	336,000	13
6	288,000	336,000	49
22	288 000	332 000	22

TABLE 2.—(*Continued*)

No.	Cornell Students Platelet Count	Colorado College Students Platelet Count	No.
30	288,000	332,000	65
38	288,000	328,000	14
59	288,000	328,000	17
75	288,000	328,000	55
85	288,000	328,000	79
89	288,000	328,000	99
96	288,000	324,000	28
99	272,000	324,000	45
70	272,000	324,000	67
60	272,000	324,000	97
4	272,000	320,000	3
8	272,000	320,000	12
37	272,000	320,000	76
97	256,000	320,000	81
90	256,000	316,000	4
83	256,000	316,000	6
56	256,000	316,000	19
53	256,000	316,000	72
45	256,000	312,000	10
14	256,000	312,000	33
18	256,000	312,000	38
25	256,000	312,000	51
33	240,000	312,000	82
43	240,000	312,000	96
58	240,000	308,000	43
26	240,000	304,000	9
54	240,000	304,000	75
92	240,000	300,000	21
40	224,000	300,000	32
63	224,000	300,000	35
98	224,000	292,000	69
5	224,000	292,000	66
87	224,000	292,000	24
10	224,000	288,000	36
15	224,000	288,000	46
19	224,000	288,000	85
44	208,000	284,000	29
13	208,000	284,000	98
31	208,000	276,000	16
46	208,000	272,000	37
81	192,000	272,000	53
41	192,000	268,000	54
94	192,000	268,000	92
39	192,000	252,000	30
76	176,000	252,000	34
86	176,000	252,000	60
17	160,000	240,000	40
	Average, 302,000 Mean... 288,000	Average, 340,000 Mean... 336,000	Pct. Inc., 12.2

Colorado Springs from sea-level and at intervals for two months thereafter.

We[13] have shown elsewhere that blood-platelets were also increased in the circulating blood by (1) carbon monoxid poisoning, (2) hyperemia of the marrow of long bones.

It has been known for some time that blood-platelets were increased in tuberculosis. Our observations both in guinea-pigs and man agree with this.

Other blood-elements are increased as in Table 3.

Tables 4 and 5 show a series of counts on healthy and tuberculous guinea-pigs.

It is especially worthy of notice that not only was a distinct increase in the leukocytes recorded in the tuberculous guinea-pigs, an average of 13,200 per cubic millimeter, as against an average of 7,620 per cubic millimeter for healthy pigs, but also a decided gain in the number of blood-platelets from an average of 438,000 per cubic millimeter in healthy pigs to an average of 622,000 in the tuberculous animals.

TABLE 3.—Other Blood-Elements in New York and Colorado

	Sea Level Percentage	Colorado Springs Percentage	Per Cent. Increase
Lymphocytes	36	43	19.3
Hemoglobin	100	110	10.0
Red Corpuscles	5,000,000	6,000,000	20.0

We have divided the counts on tuberculous patients into the following classes:

1. Early afebrile tuberculosis, 45 counts; 35 patients; 368,000 blood-platelet average.
2. Advanced quiescent tuberculosis, 50 counts; 40 patients; 412,000 blood-platelet average.
3. Advanced active tuberculosis, 26 counts; 22 patients; 496,000 blood-platelet average.

The observations on children with measles, given in Table 6, are of considerable interest.

It is at once seen that a decrease of platelets takes place during the disease.

Only further observations can determine whether the low platelet count may be connected with the high incidence of tuberculosis after measles.

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[13] Webb, Gilbert and Havens: Colorado Med., January, 1914.

TABLE 4.—BLOOD-PLATELET COUNT IN NORMAL GUINEA-PIGS AT COLORADO SPRINGS, COLO.

No.	Date	Platelets	Total Leukocytes
1	8/ 4/13	454,000	9,800
2	....	476,000	8,000
3	....	428,000	6,400
4	....	444,000	7,600
5	8/ 5/13	444,000	7,000
6	8/ 7/13	464,000	9,000
7	....	384,000	7,200
8	8/ 8/13	368,000	6,200
9	....	392,000	7,000
10	8/12/13	482,000	8,000
11	2/ 4/14	424,000	
12	2/ 4/14	476,000	
13	2/ 4/14	408,000	
14	2/ 4/14	464,000	
15	2/ 4/14	364,000	
16	2/11/14	432,000	
17	2/11/14	412,000	
18	2/11/14	424,000	
19	2/11/14	440,000	
20	2/16/14	464,000	
21	2/16/14	484,000	
22	2/16/14	436,000	
23	2/16/14	424,000	
24	2/23/14	448,000	
25	2/23/14	424,000	
26	2/23/14	528,000	
		Average 434,000	Average 7,600

TABLE 5.—BLOOD-PLATELET COUNT IN TUBERCULOUS GUINEA-PIGS AT COLORADO SPRINGS, COL.

No.	Date	Platelets	Total Leukocytes
1	7/24/13	696,000	14,000
2	7/24/13	760,000	13,000
3	7/25/13	724,000	12,000
4	7/25/13	604,000	11,000
5	7/25/13	576,000	12,000
6	8/12/13	612,000	11,000
7	8/12/13	776,000	10,000
8	8/13/13	492,000	11,000
9	8/13/13	540,000	11,000
10	8/13/13	596,000*	15,000
11	8/13/13	468,000*	12,000
		Average 622,000	Average 13,200

*Counts 10 and 11 were made direct from jugular vein under ether. All others were made by cutting the pig's ear.

It is conceded that Nature rarely operates without a purpose. Callus is not thrown out, as a rule, unless a bone is broken. In pneumonia we have generally a remarkable increase in the numbers of the polymorphonuclear leukocytes, the reason for which is better understood since Metchnikoff's studies in phagocytosis.

It has been recognized for some time that in several diseases, including tuberculosis, the blood-platelets become remarkably increased in number.

It is reasonable, therefore, to ask why do these elements increase, and do they take any part in immunity phenomena?

The microscope has never revealed their presence in the structure of tubercles, the formation of which is considered an immunity process. But other immunity processes are at work in the defense of the organism against the tubercle bacillus, for we know of the production of agglutinin, opsonin and, possibly, lysin and other immune bodies.

TABLE 6.—Blood-Platelet Count in Children Ill with Measles

Case No.	Days Since Rash Appeared	Platelet Count
1	4	248,000
1	14	520,000
2	5	292,000
2	16	348,000
3	3	260,000
3	7	300,000
4	1	288,000
4	3	276,000
5	5	260,000
6*	4	290,000

* An adult tuberculous patient. The tuberculosis pursued a rapid course following this attack of measles.

It is natural to ask, then, if Nature increases the blood-platelets in the victim of tubercle infection; is it for a purpose comparable with that found by Grüber and Futaki in rabbits or rats infected with anthrax?

Metchnikoff has maintained that antibodies, such as opsonins, are set free from leukocytes when these cells disintegrate. It is thought that serum as opposed to blood-plasma is very rich in opsonins, yet the work of Dastre[14] proves that the white corpuscles are not disintegrated when blood coagulates.

The thought occurred, therefore, can platelets furnish opsonin? The experiments shown in Table 7, several times carefully and thoroughly repeated, certainly suggest that they can. The table presents a composite picture of the results of five careful and complete experiments.

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[14] Dastre, quoted by Aynaud: Le globulin de l'homme. Ann. de l'Inst. Pasteur, January, 1911.

Similar experiments were carried out with similar results with staphylococci, so that it is not impossible that blood-platelets contain or supply opsonin.

For these and the subsequent experiments, the platelets were obtained by fractional centrifugation in citrated normal salt solution. It was well known to those who worked with the opsonic index that the fluid remaining after throwing down the corpuscles by means of the centrifuge, contained platelets. This fluid was now removed by a pipet and centrifuged at a high speed. A faint sediment was thrown down and the supernatant fluid withdrawn. This sediment was twice washed and centrifuged with normal salt solution. The final sediment was found to consist of almost entirely pure blood-platelets. We have pointed out[13] elsewhere that vagaries in the opsonic index might partly depend on the amount of platelets the washed corpuscles contained.

TABLE 7.—Composite Results of Authors' Experiments with Blood-Platelets and Tubercle Bacilli

	Polymorphonuclears	Large Mononuclears	Percentage of Empty Cells	
			Polymorphonuclears	Large Mononuclears
Serum*	1.05	0.83	26	29
Thick platelet emulsion ...	1.41	1.33	18	20
Salt solution*	0.59	0.47	37	41

* With tubercle bacilli emulsion and white corpuscues.

Experiments were undertaken in June, 1913, to ascertain if the addition of platelets to tubercle bacilli might prevent infection when inoculated simultaneously into a guinea-pig. The culture employed was later found to be dead and so the tests were worthless.

We have shown repeatedly that the subcutaneous inoculation of a definite number of very virulent bacteria (50 tubercle bacilli of a culture from which 10 bacilli have been found to cause infection), isolated by the Barber technic, will almost invariably cause infection, and such infection is always accompanied first by enlargement and infection of the adjacent lymph-nodes. We have made it a rule to inoculate always into the nipple area so that subsequent enlargement of the groin glands can be readily detected; usually after the second week.

It has frequently happened with these small doses that no local lesion has developed, but in animals so inoculated we have so far never missed the glandular involvement when the pigs have later been found tuberculous at necropsy.

The following experiments would, therefore, seem unusual:

Four young guinea-pigs were inoculated in the right nipple area.

Pig 1, weight 255 gm., received 200 tubercle bacilli.

Pig 2, weight 212 gm., received 200 tubercle bacilli + platelets from normal pig after incubation one-half hour.

Pig 3, weight 214 gm., received 200 tubercle bacilli.

Pig 4, weight 183 gm., received 200 tubercle bacilli + platelets from normal pig after incubation one-half hour.

Air was blown in following the injection in all except Pig 3. Forty-two days later Pig 1 was killed. No local tuberculous lesion; glands enlarged in right groin; extensive infection of spleen; mediastinal glands infected; extensive disease in liver. Thirty-four days later Pig 2 died of epidemic intestinal disease. One caseous nodule was found in the spleen. Nothing else was found, no local lesion and no groin glands infected. Forty-four days later Pig 3 was killed. No disease was found. It is probable that the bacteria were not safely inoculated. Forty days later Pig 4 died. No local lesion. No local glands in groin involved; extensive tuberculous infection in liver and spleen and mediastinal gland enlarged.

The chief interest of this experiment would seem to be in the absence of infection of the groin glands in Pigs 2 and 4.

This absence of local glandular infection is most unusual and possibly might suggest a sensitizing of the bacilli by the platelets allowing a more general distribution in the system.

We have found in earlier experiments that 60 bacilli of this virulent culture will produce rapid and wide-spread lesions in such young guinea-pigs as well as infection of the glands adjoining the site of inoculation.

The following experiments deal with older guinea-pigs, usually full-grown. The injection in all animals was made in the right nipple area.

Dec. 29, 1913: Pig 53; 200 tubercle bacilli. Pig 54; 200 tubercle bacilli with human platelets. Pig 55; 200 tubercle bacilli with human platelets and inactive serum. Pig 56; 200 tubercle bacilli with active (human) serum.

Jan. 18, 1914: Pig 55 has enlarged hard gland right groin. Others negative.

Feb. 16, 1914: Pig 56 has enlarged hard gland right groin.

April 11, 1914: Pig 55, killed. Enormous tuberculous liver and spleen; lungs completely involved (local lesion and glands had been previously removed).

April 23, 1914: Pig 53 gave birth to two young three weeks ago. Killed; no tuberculosis discovered. It is probable the bacilli were not safely inoculated.

April 23, 1914: Pig 56 gave birth to two young two weeks ago. Killed. Tuberculous spleen, few tubercles in liver, local gland, mediastinal gland.

April 17, 1914: Pig 54 has not yet shown infection. It is possible that bacilli were not safely inoculated. Fifty bacilli of same culture were now inoculated into left nipple area and also into a control, Pig 86.

May 23, 1914: Pig 54, killed. Spleen markedly tuberculous, bronchial gland and deep inguinal gland, left side affected. No local lesion. Pig 86, killed. Spleen and bronchial glands affected. No local lesion.

This last experiment indicates that if the 200 bacilli were originally safely inoculated, immunity was not produced sufficiently to protect the pig later against 50 tubercle bacilli.

The foregoing experiments were repeated with the same virulent culture, but instead of human blood-platelets, "immune" platelets from another pig and serum of this pig were employed.

Jan. 12, 1914. Pig 57, weight 565 gm.; 200 tubercle bacilli injected. Pig 58, weight 555 gm.; 200 tubercle bacilli injected, + platelets taken from a guinea-pig with advancing tuberculosis. Pig 59, weight 437 gm.; 200 tubercle bacilli injected; serum unheated; platelets taken from a guinea-pig with advancing tuberculosis. Pig 60, weight 513 gm.; 200 tubercle bacilli; serum heated to 55 C. for one-half hour; platelets taken from a guinea-pig with advancing tuberculosis.

All the pipets were incubated with their contents one-half hour before inoculations were made.

Feb. 7, 1914: Pig 60, large gland palpable in right inguinal region.
Feb. 12, 1914: Pig 57, large gland palpable in right inguinal region.
Feb. 14, 1914: Pig 59, large gland palpable in right inguinal region.
Feb. 16, 1914: Pig 58, no enlarged glands palpable.

April 3, 1914: Pig 57, local inguinal glands stationary or smaller. Pig 58, no local enlarged glands. Pig 59, local glands increasing in size. Pig 60, local glands very large.

April 8, 1914: Pig 57, found dead; extensive tuberculosis of liver and spleen; large mediastinal glands; local glands had diminished in size recently and no tuberculosis was found.

April 25, 1914: Pig 59, killed. Extensive tuberculosis of spleen; moderate infection of lungs and liver; mediastinal glands very large; local inguinal glands enlarged.

April 25, 1914: Pig 60, killed. Moderate tuberculosis of spleen. Scattered nodules in lungs and liver. Mediastinal glands very large. Local inguinal glands palpable.

May 16, 1914: Pig 58, found dead. No tuberculous lesion anywhere. Death due to epidemic gastro-enteritis. It is, of course, again possible that the bacilli were not safely inoculated.

We realize that not enough experiments have yet been carried out and that the work is incomplete. We may, however, consider that:

1. It is suggestive that when a small amount of a thick emulsion of platelets is added to a lethal dose of virulent tubercle bacilli, infection would seem to be either modified or prevented.

2. When serum is added to this mixture, no evidence can be shown of any change in the infection produced from that of the controls.

CONCLUSIONS

1. Blood-platelets are consistently increased in the tuberculosis of man and guinea-pigs.

2. At an altitude of 6,000 feet, blood-platelets are increased in the circulating blood of man and guinea-pigs.

3. Blood-platelets would appear either to contain or to supply opsonin.

4. Blood-platelets added to a lethal dose of tubercle bacilli appear to modify the course of infection or to prevent it.

5. The addition of serum to a mixture of blood-platelets and tubercle bacilli appears to interfere with this modification.

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PULMONARY PHYSICAL SIGNS AND ROENTGEN-RAY FINDINGS IN HEALTHY ADULTS *

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The general campaign against tuberculosis, and particularly the influence of tuberculosis sanatoriums, has kindled an eager interest in the early diagnosis of pulmonary tuberculosis. Treatment, to be availing, must be begun before the presence of moist râles in the lungs and tubercle bacilli in the sputum proclaims the destruction of tissue. The various biological tests, hypersensitiveness, complement fixation, agglutination, etc., that have lent such valuable aid in the diagnosis of other infectious diseases have failed in tuberculosis, because in tuberculosis, contrary to the conditions in other infections, infection and disease are not synonymous terms. Therefore, effort has been directed toward refining fundamental diagnostic methods. The symptomatology of tuberculous disease has been more thoroughly analyzed and many valuable symptomatic relations have been established. It is only exceptionally, however, that a diagnosis can be made from the symptoms alone, since similar symptoms and symptom-groups occur in many other conditions. In most instances a satisfactory diagnosis is reached only when symptoms are supported by the presence of pulmonary physical signs.

The physical examination having become the diagnostic arbiter in nearly all doubtful cases of pulmonary tuberculosis, the arts of percussion and auscultation have been cultivated assiduously and to-day are practiced with heretofore unequalled precision. Slight lesions are discovered with remarkable accuracy as control Roentgen-ray examinations have demonstrated.

ABNORMAL PHYSICAL SIGNS

Since slight abnormalities in the pulmonary physical signs have such an important bearing on the early diagnosis of pulmonary tuberculosis, therefore, it is highly important to be acquainted with all the conditions under which such slight abnormalities may occur. Otherwise, we are not in a position to give them their proper diagnostic value in specific instances.

For many years we have been impressed, in making routine physical examinations of a large number of students and nurses, by the frequent

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occurrence of more or less marked apical abnormalities. On a number of occasions we have emphasized this experience in discussing the value of pulmonary examinations in the diagnosis of pulmonary tuberculosis. The matter has seemed to us of enough importance to make it desirable to have this experience in precise form, and with this object in view we undertook to study, with unusual care, the pulmonary physical signs in fifty healthy young adults.

For the purpose of this study we selected fifty students and physicians who, as far as symptoms go, were in perfect health. A minute examination was made of the chest by Dr. Hamman, and the results of inspection, palpation, percussion and auscultation were recorded and the deduction made therefrom as to the condition of the lungs committed to writing. A roentgenogram was then finished and reported on in writing by Dr. Baetjer, who had no knowledge of the outcome of the physical examination. Finally, the results of the physical examination and of the Roentgen-ray examination were compared.

The great difficulty in the way of satisfactorily appreciating slight deviations from the normal in pulmonary examinations is the absence of any concrete standard for comparison. The following brief statement of every-day observations illustrates what is meant.

When one looks at a well-developed man the chest is seldom seen to be quite symmetrical. Nearly always one clavicle is a little more prominent than the other, the corresponding shoulder a little lower, the upper dorsal vertebrae a little curved toward the opposite side, and on quite breathing expansion of the upper portion of the chest on that side is diminished. In right-handed individuals the changes are present on the right side, in left-handed individuals on the left side. The degree of difference varies greatly under what must be considered normal conditions, and there is no set limit to divide accurately the normal from the abnormal. If such changes are well marked on the side more used, they suggest the presence of pulmonary involvement on that side; if they are present on the opposite side, they point much more strongly to the presence of a lesion there.

Fremitus is a coarse sign of inestimable value in studying gross pulmonary lesions but lends little assistance in the discovery of slight changes. The quality of the voice and the character of the chest wall modify fremitus to such an extent that there is the widest individual variation. The sign is of value only comparatively between the two sides of the chest in the same individual. Such comparisons must take into account the normal accentuation of fremitus throughout the right side, particularly over the right upper. The difference is not a constant ratio, but varies greatly among individuals, and the difference must be very marked before we are justified in attaching to it diagnostic significance. When fremitus is felt better over the left upper than over the right upper, there is usually a lesion in the left upper lobe to explain the anomaly, although occasionally this difference exists in the absence of any other evidence of pulmonary abnormality.

In percussion we are forced again to a comparison of the note and sense of resistance in corresponding areas of the two sides of the chest. There is no absolute normal note with which to compare, the character of the note depending so largely on the thickness of the chest wall and the configuration of the chest. Our notion of the normal percussion note is a composite impression derived from a long and arduous experience in percussing innumerable chests. It is not an objective standard that we can demonstrate and measure by, outside of such experience. It is usually stated that the percussion note is a little less resonant above the right clavicle and in the right supraspinous fossa than at corresponding points on the left. We are not convinced that this difference exists normally to very light percussion. If a heavier percussion stroke is used the air in the trachea, which lies much nearer to the right than to the left apex, is thrown into vibration and the note is thereby modified.

The barriers in the way of standardizing physical signs are most obvious in considering auscultation. There is the greatest variation in the breath and voice sounds of normal individuals. In some, the breath-sounds are unusually loud, in others, unusually distant and faint; in some, expiration is more prominent at the apices than in others, and so on. Forced to use comparison of the intensity and quality of the breath and voice sounds at corresponding areas on the two sides, we face the difficulty raised by the normal difference between the two sides, particularly the well-marked difference at the apices. As in percussion, so in auscultation, there is no absolute normal and we must adopt an arbitrary standard that is born of individual experience.

These obvious difficulties are mentioned to emphasize the statement that this study is offered merely as a personal contribution to an interesting subject. What is found in the lungs on physical examination will vary with skill and experience, purely personal qualities, which give a strong individual flavor to the result. Still, we are convinced of the accuracy of our general conclusions and believe they are verifiable, although no two men might concur in detail about the interpretation of the physical examinations. We wish to emphasize further that we have sought for the very slightest deviation from normal in the physical signs, deviations that in routine pulmonary examinations we would disregard. But this will appear plainly enough in the illustrations we will furnish.

Having in mind the exception that might be taken to deductions based on examinations which we freely admit lack a desirable standardization, we sought to give them strength through the support of Roentgen-ray examinations. Roentgenograms are certainly objective, and though their skilled interpretation requires experience, still the data on which the conclusions rest can be satisfactorily demonstrated and are incontrovertible. A comparison of the results of the physical examination and the Roentgen-ray control must therefore reveal points of great interest and importance.

In reading the Roentgen-ray plates the following points were noted:

1. The density of the hilus shadow, which is recorded as normal, slightly increased, moderately increased or markedly increased.

2. The number and position of gland shadows.

3. The bronchial tree shadow (which includes bronchi, vessels and connective tissue), which is recorded as normal, slightly increased, moderately increased or markedly increased, and differences in various locations described.

4. The density of the lung substance itself. Special attention was given the condition of the apices and indeed abnormalities were limited mostly to the apices. On Roentgen-ray examination, the normal lung apices are clear and the apical spaces are equal on the two sides. The earliest change occurring at the apices is invasion by fine radiating lines extending from the bronchial tree, subdivisions which become visible only as the result of inflammatory changes. Following this the whole apical region becomes a little cloudy and opaque, probably due in large part to pleural changes. Finally, contraction may occur, which is early evidenced by narrowing of the space between the first and second ribs.

Regarding the Roentgen-ray findings as a control of the physical examination, the fourth point alone comes into consideration. Mediastinal shadows, though dense, give no physical sign. Glands enlarged sufficiently to cast definite shadows to Roentgen-ray illumination are still quite beyond the reach of percussion and auscultation. Increased density of the bronchial tree is discoverable only when the increase is unusually marked and extends close to the surface of the lungs in the region of the apices.

Reviewing the results of the physical examination we find that of the fifty healthy adults examined, in eleven the lungs are recorded as practically normal. We say practically normal, because in only five the note was made that absolutely no abnormality was found; in the remaining six very slight changes in the physical signs were discovered, but the changes were so slight that they were considered questionable. The five perfectly normal cases all had Roentgen-ray examinations. In three the note is made, "Perhaps slight clouding at the right apex, but practically normal;" one showed very slight clouding at both apices; one, slight clouding and retraction at the left apex. An illustrative case is the following:

Case 1.—Medical student, aged 23; weight 168 pounds; height 5 feet 11½ inches. No tuberculosis in the family. Always strong and healthy. Measles and mumps in childhood. Typhoid fever at 15 years of age. No pulmonary infections. Not subject to colds.

Well-nourished man of healthy appearance. Chest is well formed. Right clavicle a little more prominent than the left. Right shoulder sags a little. Viewed from in front, the right seems to move more than the left, but viewed from behind the left shoulder moves distinctly a little more than the right. (Right-handed.) Fremitus is better felt on the right in about the normal proportion. The percussion note is everywhere resonant, no definite difference between the two sides. Breath sounds are everywhere vesicular and clear-cut. Whispered voice sounds show the normal relation.

Apical resonance zone: right, 7 cm.; left, 7 cm. Diaphragm movements (difference of lung border on full inspiration and forced expiration), right, 9.5 cm.; left, 9.5 cm. Circumference of chest: at rest, 93 cm.; full inspiration, 99 cm.; full expiration 90 cm.

Impression: A normal chest.

Roentgen-ray plate 16,538. Moderately increased density of the hilus shadow on both sides. Numerous small fibrous glands on both sides. Slight thickening of the bronchial tree throughout on both sides. Perhaps very slight clouding at the right apex.

Of the six practically normal chests with slight, questionable physical signs, five had Roentgen-ray examinations. In all five physical examination indicated slight changes at the right apex, whereas Roentgen-ray examination showed normal lungs in one instance, slight changes at the left apex in three and slight changes at the right in one. The following is an illustration from this group.

Case 2.—Medical student, aged 22; weight 158½ pounds; height 5 feet 10¼ inches.

No tuberculosis in the family. Has always been strong and healthy. Measles, chicken-pox, mumps and pertussis in childhood. No pulmonary disease. Not subject to colds or bronchitis.

Well-nourished man of healthy appearance. The chest is well formed. Right clavicle is a little more prominent than the left. Some restriction of movement over the right upper, front and back. The lower right moves a little more than the left. (Right-handed.) Fremitus is more marked on the right than on the left and the difference is perhaps a little greater than normal. The percussion note is a little different at the two apices, but it is difficult to say which side is abnormal. The impression is that there is a little impairment above the right clavicle and in the right supraspinous fossa. The breath sounds are clear cut and vesicular; they are unusually well heard on both sides; a little harsh and a little blowing above the right clavicle and in the right supraspinous fossa. The whispered voice sounds are well transmitted on both sides, the intensity on the right being a little greater than is usually heard. After coughing, a few transient crackles are heard above the right clavicle. A. r. z.: right, 6.5 cm.; left, 6.5 cm.; diaphragm movements in midaxillary line; right, 8.5 cm.; left, 8.5 cm. Circumference of the chest at the nipples: at rest, 83 cm.; full inspiration, 89 cm.; full expiration, 82 cm.

Impression: The chest is normal except for the slight changes at the right apex and these are questionable. The percussion note is perhaps a little impaired and the breath sounds a little more intense than they are usually heard.

Roentgen-ray plate 16,604. There is very slight increased density of the hilus shadow on the right side. No glands visible. The bronchial tree shows a normal degree of density. There is very slight clouding and definite, though slight retraction of the left apex. Diaphragm is normal. Heart pendulous.

Of the fifty examinations, in eighteen, definite abnormalities were found at the right apex; in fifteen, though definite, the abnormalities were slight, in three they were well marked. Of the fifteen with slight though definite changes in the physical signs the Roentgen-ray results accorded in detail with the physical examination in seven instances; in two instances slight changes were found at the left apex as well as at the right; in three the right apex was found clear, though there was a little clouding at the left apex; in two no definite changes were discovered in the lung; one had no Roentgen-ray examination.

The following is an illustration of agreement in the results of the physical and Roentgen-ray examinations.

Case 3.—Medical student, aged 23; weight 133 pounds; height 5 feet 8½ inches. No tuberculosis in family. Always strong and healthy. Mumps, measles, pertussis and croup in childhood. No pulmonary infections.

Well nourished, good color, healthy appearance. Chest is well formed; right clavicle a little more prominent than the left. The right shoulder sags somewhat. Movement a little restricted over the right upper. (Right-handed.) Fremitus better felt on the right than on the left in about the normal proportion. Percussion note definitely a little impaired above and below the clavicle and in the supraspinous fossa on the right. Above the right clavicle inspiration is obscured and distant, whereas expiration stands out prominently and is prolonged and blowing. The same relation exists but to a less marked degree below the clavicle and in the supraspinous fossa. On the left breath sounds are normally vesicular and clear cut. The voice sounds are better transmitted above and below the clavicle and in the supraspinous fossa on the right than is usual.

Apical resonance zone: right, 6 cm.; left, 7 cm. Diaphragm movements: right, 8 cm.; left, 8 cm.

Roentgen-ray plate 16,609. Moderate increased density of the hilus shadow on both sides, more marked on the right. No enlarged glands. Moderate increased density of the bronchial tree generally, more marked on the right than on the left. Slight clouding of the right apex above the clavicle. The right side of the diaphragm is a little irregular in outline.

The following instance illustrates disagreement between the physical and Roentgen-ray examination. It is possible that the spinal curvature may have caused the change in the physical signs.

Case 4.—Medical student, aged 27; weight 147 pounds; height 5 feet 8¼ inches. No tuberculosis in the family. Always strong and healthy. Measles and pertussis in childhood. No serious illness since childhood. Rather subject to colds. Has chronic catarrh of nose and throat.

Fairly well nourished. Healthy appearance. Marked retraction of the right side; the whole side apparently smaller than the left. Right clavicle more prominent than the left, with marked retraction below the clavicle. Right shoulder droops and there is slight curvature of the spine to the left in the upper dorsal region. The scapula is low. Marked deficiency of movement on the right side. (Right-handed.) Fremitus is better felt on the right than on the left in about the normal relation. The percussion note is a little impaired above and below the clavicle on the right and perhaps a little impaired above the clavicle and in the supraspinous fossa on the left. Inspiration above the clavicle and in the supraspinous fossa on the right is a little obscure and expiration a little blowing. Above the clavicle and in the supraspinous fossa on the left breath sounds are a little harsh. The whispered voice sounds are more accentuated than normally above the clavicle and in the supraspinous fossa on the right.

Apical resonance zone: right, 6 cm.; left, 6 cm. Diaphragm movements: right, 8 cm.; left, 8.5 cm. Circumference of the chest at rest, 84 cm.; full inspiration, 91 cm.; full expiration, 82 cm.

Impression: Definite changes at the right apex and perhaps slight changes at the left.

Roentgen-ray plate 20641. Slight increased density of the hilus shadow on both sides. No glands. Very slight thickening of the bronchial tree on both sides. The lungs are normal. The diaphragm is a trifle higher on the left than on the right.

Of the three instances with more marked abnormalities at the right apex, two had Roentgen-ray examinations. In one the Roentgen ray and physical examinations agree in detail, in the other the Roentgen ray reveals a slight lesion on the left, besides the more marked change at the right. The latter instance is briefly as follows:

Case 5.—Medical student aged 22 years; weight, 160½ pounds; height, 6 feet ½ inch. No tuberculosis in the family. Has always been strong and healthy. Chicken-pox, measles and pertussis in childhood. Several attacks of bronchitis, one during the last few weeks.

Well nourished, good color, healthy appearance. Chest well formed, the right clavicle a little more prominent than the left. Slightly less movement over the right upper. (Right-handed.) Fremitus better felt over the right in about the normal proportion. Percussion note definitely impaired above the clavicle and less markedly so below the clavicle and in the supraspinous fossa on the right. Above the right clavicle inspiration is harsh and expiration prolonged and blowing; below the clavicle and in the supraspinous fossa expiration is a little more blowing than normally. Whispered voice sounds are better transmitted on the right than is usual. A few fine transient râles above the right clavicle.

Apical resonance zone: right, 6.5 cm.; left, 7 cm. Circumference of chest: at rest, 85 cm.; full inspiration 91 cm.; full expiration, 82 cm.

Impression: Definite changes at the right apex.

Roentgen-ray plate 16660. Moderate increased density of the hilus shadow on both sides. Few small fibrous glands on the right. Moderate increased density of the bronchial tree more marked on the right than on the left and particularly well marked in both upper lobes. Slight clouding of both apices, more evident on the right than on the left.

Twelve of the fifty examinations showed definite changes in the physical signs at the left apex; in eight instances the changes, though definite, were not marked; in four they were well marked. In the eight instances with definite though slight changes, the Roentgen-ray examination agrees accurately with the physical examination in three;

in four it reveals a slight lesion at the right as well as left; in one the left apex is found clear, the right a little cloudy.

In the four instances of more marked changes at the left apex the Roentgen-ray examination agrees accurately, except that in one slight clouding is present as well at the right. As an illustration of this group, the following instance is cited:

Case 6.—Physician, aged 27; weight, 162 pounds; height, 5 feet 11 inches. No tuberculosis in the family. Lobar pneumonia involving the right lower lobe five years ago. Typhoid fever eight years ago. Has mild attacks of bronchitis about once a year, the last attack about four months ago.

Well-nourished man of healthy appearance. Chest well formed, symmetrical. Clavicles a little prominent, the left a little more prominent than the right. The left shoulder sags a little. There is very slight curvature of the spine to the right in the upper dorsal region. Movement is diminished over the left upper. (Right-handed.) The fremitus is better felt on the right than on the left but the difference between the two sides is not as marked as is usual. The percussion note is a little higher pitched above the left clavicle elsewhere it is perfectly resonant and equal on the two sides. Inspiration above and below both clavicles is rather obscure and not as clearly vesicular as normally. Above the left clavicle inspiration has lost its sharp, crisp quality; in the left supraspinous fossa breath sounds are diminished in intensity. Above and below the clavicles and in the supraspinous fossa the whispered voice sounds, though better transmitted on the right than on the left, do not show in this respect as great a difference as is usual. After coughing there are a few transient crackles above and below the left clavicle.

Apical resonance zone: right, 6 cm.; left, 6 cm. Diaphragm movements: right, 8.5 cm.; left, 8.5 cm. Circumference of chest: at rest, 86.5 cm.; full inspiration, 92.5 cm.; full expiration, 84.5 cm.

Impression: The physical signs point toward a definite though slight lesion at the left apex.

Roentgen-ray plate 18163. Markedly increased density of the hilus shadow on both sides, especially on the right. A few small fibrous glands on the right side in the second and third interspaces. Bronchial tree shows slight thickening throughout both sides a little more marked on the left than on the right in upper lobe. There are definite small patches of fibroid tissue scattered throughout the right lower lobe. The right apex, however, is clear. There is definite clouding of the left apex. Diaphragm normal.

Comment: In this case the results of the physical examination and the Roentgen-ray examination almost completely coincide; perhaps the marked increased density of the bronchial tree in the right lower lobe may be the remains of the pneumonia five years ago.

In nine of the fifty cases there were abnormalities in the physical signs at both apices; in six the changes were definite though slight, in three very well marked. In the six with slight changes the Roentgen ray and physical examinations coincide in three; whereas, in the remaining three the Roentgen ray showed changes at the left apex alone. Of the three with well-marked changes two had Roentgen-ray examinations and in both they correspond accurately with the results of physical examination. These two individuals showed more marked changes on both physical and Roentgen-ray examination than any others in this series and a brief account of the findings follows:

Case 7.—Physician, aged 42; weight, 117 pounds; height, 5 feet 3½ inches. Mother died of tuberculosis when patient was 6 years old; one brother has had tuberculous pleurisy. Has always been healthy, but never robust. Had diphtheria at 6 years, no serious illness since. Several attacks of tonsillitis. Bronchitis at age of 19. No serious illness. Not subject to colds.

A small man of delicate appearance. Left clavicle a little lower than the right; viewed from in front the left side moves a little more than the right; viewed from behind the right shoulder moves definitely more than the left. (Right-handed.) Fremitus is better felt on the right than on the left side and the difference is greater than is usually present. The percussion note is definitely impaired above and below the right clavicle and in the right supraspinous fossa; there is definite but not marked impairment above the left clavicle. Above the right clavicle inspiration is distant and obscure; expiration is distant but definitely blowing in quality. On deep breathing inspiration is harsh and roughened above and below the clavicle and expiration quite blowing. In the right supraspinous fossa inspiration is harsh and halting; expiration blowing in quality but not accentuated. Above the left clavicle and in the left supraspinous fossa breath sounds are diminished in intensity, on deep breathing inspiration is very harsh. The whispered voice sounds are very definitely increased in intensity above and below the clavicle and in the supraspinous fossa on the right; they are also unusually well heard in the left supraspinous fossa. After coughing a few fine râles are heard above and below the right clavicle which subsequently disappear; and a few transient râles in the left supraspinous fossa.

Apical resonance zone: right, 5.5 cm.; left, 6.5 cm. Diaphragm movements: right, 6.5 cm.; left 7.5 cm. Circumference of chest: at rest, 75 cm.; full inspiration, 84 cm.; full expiration, 74 cm.

Impression: There is a very definite lesion in the right upper lobe and a definite though less extensive lesion in the left upper lobe.

Roentgen-ray plate 21079. Markedly increased density of the hilus shadow on both sides. Small number of fibrous glands on both sides. There is marked thickening of the bronchial tree throughout both lungs and especially marked in both upper lobes. Marked clouding of both upper lobes with retraction, especially well marked over the right upper lobe. Three small cavities in the right upper lobe and one in the left. Diaphragmatic adhesions on both sides.

Case 8.—Student, aged 22. Weight, 127¾ pounds; height, 5 feet 6 inches. Father, mother, five brothers, all living and well. Two paternal aunts died of tuberculosis. Has always been well except for throat trouble; voice is often hoarse; clears throat frequently. Severe diphtheria in childhood. At the age of 10 and again at the age of 14 had severe grip which affected the throat. Measles mumps and pertussis in childhood. General health has always been good.

Fairly well-nourished man; skin a little sallow. Mucous membranes good color. The chest in general is well formed. The right clavicle is a little more prominent than the left. The right shoulder sags a little; slight limitation of movement over the right upper. (Right-handed.) Fremitus is better felt on the right than on the left side; indeed it is exaggerated on the right side. There is definite impairment of the percussion note above and below the right clavicle and in the right supraspinous fossa. Above and below the right clavicle inspiration has almost completely lost its vesicular quality and is very blowing, and expiration is intensely tubular in quality. The same changes are present in the supraspinous fossa, but not so marked as in front. Above and below the left clavicle expiration is better heard than normally, but inspiration is clear-cut and vesicular. Whispered voice sounds are markedly increased in intensity above the right clavicle and to a less extent below the clavicle and in the supraspinous fossa. No râles.

Apical resonance zone: right, 6.5 cm.; left, 6.5 cm. Diaphragm: right, 7.5 cm.; left, 7.5 cm. Circumference of chest: at rest, 77 cm.; full inspiration, 84 cm.; full expiration, 75.5 cm.

Impression: A very definite and well-marked lesion in the right upper lobe; also a slight lesion in the left apex.

Roentgen-ray plate 20091. Markedly increased density of the hilus shadows, especially marked on the right. A few small fibrous glands on the right. Marked thickening of the bronchial tree especially below both clavicles; more marked on the right than on the left. There is marked clouding above and below the right clavicle but no retraction; slight clouding at the left apex without retraction. Diaphragm normal.

To state the results briefly: A careful physical examination of the chest of fifty healthy adults shows the pulmonary physical signs to be practically normal in eleven, definitely though not markedly altered in thirty, more obviously altered in seven and greatly changed in two. That the changes in the physical signs are the expression of corresponding changes in the lungs is borne out by the results of the Roentgen-ray examinations. Forty-six of the series had roentgenograms taken and in only six were the apices found to be normal.

The results of the physical and Roentgen-ray examinations, although agreeing in a large proportion of the cases, still not infrequently are at variance. In twenty-two of the forty-six cases the agreement is complete. The disagreement in the remaining twenty-four cases is as follows:

In six instances the physical examination shows practically normal conditions, whereas the Roentgen-ray examination shows slight apical clouding. This source of disagreement scarcely needs comment. To give such slight changes in the physical signs as are under consideration, a small lesion must be favorably located. A small lesion near the surface will give more obvious physical signs than a larger lesion more deeply situated, whereas the Roentgen-ray examination will reveal the condition of the apex as a whole.

In eighteen instances the physical examination indicates a definite lesion at one apex, whereas the Roentgen-ray examination shows clouding at both apices. This is hardly a serious disagreement.

In four instances the physical examination indicates a slight lesion at one apex whereas the Roentgen-ray examination shows clouding at the opposite apex. In all four instances the changes in the physical signs were very slight. One is at times sure that there is a little difference in the percussion note at the two apices, though it may be impossible to say which apex is normal and which changed. Also it is difficult at times when the breath-sounds vary to be sure that they are exaggerated at one apex rather than diminished at the other.

In two instances the physical examination indicates slight abnormalities at one apex whereas the Roentgen-ray examination shows both apices to be clear. One such instance is cited in the text, the difference in the physical signs being due, we believe, to the lateral scoliosis.

In four instances the physical signs indicate a lesion at both apices, whereas Roentgen-ray examination reveals clouding at only one apex. It is interesting that in all four instances the Roentgen-ray examination shows the lesion to be at the left apex.

Regarding the nature of the alterations in the lungs that occasion the changes in the physical signs and in the Roentgen-ray shadows, we are not prepared to make a definite statement. The increase in the hilus shadows, the glands and the deepening of the bronchial tree shadows, are regarded as evidence of old infections. No doubt tuberculous infections are frequently the cause, but tuberculosis leaves no specific marks and experience has convinced us that other infections, bronchitis, pneumonia, influenza, etc., may cause such changes and are indistinguishable from those produced by tuberculosis. The changes at the apices, infiltration, fibrosis and pleural thickening, we consider from general evidence to be nearly always the results of old quiescent tuberculous infections. The frequency with which such apical abnormalities are discovered in children under 12 years of age lends support to the view.

The important bearing that the facts here brought out have on the early diagnosis of pulmonary tuberculosis needs but hasty comment. That a large proportion of healthy young adults display evident apical abnormalities must have its weight in considering the value of such signs in diagnosis. Osler aptly compares slight abnormalities in the pulmonary physical signs with the systolic murmur commonly heard at the apex of the heart. When interest in the physical signs of cardiac disease was in the period of early enthusiasm an apical systolic murmur meant mitral insufficiency. Now that long experience has given these observations great precision, mitral insufficiency (meaning an organic mitral lesion) is not so lightly diagnosed on the isolated evidence of an apical systolic murmur. This does not imply that the presence of a systolic murmur is to be ignored, for it still has an important place in diagnosis, but it means that we have learned gradually to give it its just value as a part of the whole clinical picture. For instance, in acute rheumatic fever endocarditis occurs frequently and as it attacks the mitral valves more commonly than the others we often hear a systolic apical murmur as an early indication of mitral damage. However, experience has taught us that systolic apical murmurs are commonly heard in febrile conditions when there is not the slightest reason for suspecting the presence of mitral endocarditis. Therefore, when in the course of rheumatic fever we observe an apical systolic murmur we immediately think of the possibility of mitral endocarditis, but we do not affirm its presence. The diagnosis between a so-called "functional" and a so-called "organic" mitral regurgitation depends on the evidence offered by the complete examination and the clinical symptoms. Indeed, with all available data at hand the differentiation is not always possible. Exactly analogous conditions exist in the relation of slight pulmonary apical abnormalities to the diagnosis of pulmonary tuberculosis. As isolated facts they by no means justify a diagnosis of pulmonary tuberculosis, although they acquire important value when supported by other evidence.

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CLINICAL STUDIES IN CAFFEIN *

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The pharmacological action of caffein as it relates to the normal man and animal has been for many years the subject of extensive research. Its main effects on the normal cardiovascular and nervous systems are well known. Briefly stated for the purpose of this paper, this effect of caffein in moderate doses is, first, to produce a moderate rise of blood-pressure due to a stimulant action on the heart muscle (this rise is maintained in spite of a mild degree of vasodilatation); second, an increased urinary output due to circulatory changes, and third, an increased respiratory rate. The cerebral functions are stimulated and the muscle tone increased. This experimental field was reviewed and the varying results of the different investigators harmonized by Sollmann and Pilcher[1] in 1911.

With this action in mind the pharmacologists have given the drug a high therapeutic rating and it has been extensively used as a cardiac and nerve stimulant. It is one thing to inject a drug directly into the blood-stream of an animal with a normal circulatory system or again into an animal under the influence of an anesthetic and the surgical procedures necessary for the recording of blood-pressure, variations in the size of the kidney, etc.; and quite a different thing to give a drug by mouth, in the usual clinical way, where the absorption is more or less delayed and in cases in which the cardiovascular system is not intact. In one case the results may be prompt, definite, limited; in the other case they may be slow to appear, indefinite, and continued

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* From the Medical Clinic of Prof. C. F. Hoover, Lakeside Hospital and Western Reserve University.

* An abstract of this paper was read at the Philadelphia meeting of the American Society for Pharmacology and Experimental Therapeutics, December, 1913.

* This investigation was undertaken at the suggestion of the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Sollmann, T., and Pilcher, T. D.: The Actions of Caffein on the Mammalian Circulation, Jour. Pharm. and Exper. Therap., 1911, iii, 19.

in their action. In the animal the distressing symptoms caused by the drug are beyond the reach of the investigator to determine; in the sick man these assume paramount importance. With the idea of further testing the effect of caffein on the pathological circulatory system of the human being, and if possible adding to our clinical knowledge of the drug, these observations were made.

The patients chosen were all adults, the youngest 24, the oldest 56, suffering from advanced myocardial disease. In some cases the myocardial insufficiency was primary, in others secondary to a chronic nephritis or an old endocarditis. The fifteen cases reported were selected because in these cases the observations were fairly complete. In numerous other cases the loss of specimens, death, or intercurrent complications requiring the use of other therapeutic measures made the analysis of caffein action impossible. Every case showed some sign of broken compensation at the time of the experiment, and thirteen gave evidence of retained body fluid. Dyspnea was present in 14 cases; edema of the extremities in 13 cases; ascites in 10 cases; hydropericardium and hydrothorax, single or double, in 6; tender pulsating liver in 6. Other common symptoms were cough, frontal headache, weakness, dizziness and substernal pain.

All patients were kept in bed on a constant diet. Milk with the possible diuretic action of its milk-sugar was eliminated in all but one case, and here milk had been a constant factor in the diet for several days previous to the administration of the drug. No effort was made to reduce the salt intake. The fluid intake was measured and in eleven of the fifteen cases was kept constant at amounts varying in the different cases from 930 to 2,100 c.c., depending on the wish of the patient. The patient was allowed to use what water he wanted on the first day, and that amount was taken as a constant for subsequent days. The average intake was 1,200 c.c. No medication or depleting therapy of any kind was allowed for four days preceding the experiment, so that rest as a factor in restoring cardiac function might come as near as possible to a constant level.

In eight cases citrated caffein was used in doses of 10 grains, four times daily. In Case 2, in which there was no noticeable result with that dosage, it was pushed to 100 grains a day. In two cases the dose was reduced to 5 grains, three times a day. The average of the eight cases was 32.5 grains a day of the citrate, which is the equivalent of 16.25 grains of the pure caffein. In seven cases the pure alkaloid, caffeina, was used in doses of 3 to 5 grains, four times daily; an average of 17.7 grains per day. The drug was discontinued usually because of the appearance of unpleasant symptoms. The average use of the citrate extended over 4.8 days, that of the pure drug, 4.6 days, so that both the dosage and the length of time during which the different preparations was used were practically the same.

Effect on the Pulse-Rate.—The pulse was taken one-half hour before and again one-half hour after the administration of the drug. The rate previous to giving the drug was taken as the gauge of a permanent drug effect, and in this series of observations showed no definite change. In six cases there was a permanent rise of five beats a minute, or 7 per cent., but in eight others there was a drop of 7 per cent., so that these variations fall well within the limits of chance disturbances. In one case an intercurrent febrile attack sent the pulse-rate up, invalidating the result. The maximum effect on the pulse-rate appeared in one-half hour and consisted of a temporary rise in every case, varying from 2 per cent. in Case 6 to 5.7 per cent. in Case 10. The average rise was 3.6 per cent. This rise, it is true, is relatively small, but in this series constant and for that reason worth noting. In none of these cases was there a tachycardia, the pulse ranging from 70 to 90. This fact combined with the relatively small temporary rise made it impossible to make any clinical comments on the fact noted in dogs that caffein causes a greater quickening in hearts which have a high rate. Certainly we may say that in this group of cases caffein has no permanent or clinical effect on the heart-rate.

Effect on the Respiratory Rate.—This was determined, as was the pulse-rate, by observations one-half hour before and after the administration of the drug, a total of eight readings a day. Even in cases with dyspnea and the relief of that symptom, the change in the rate could not be ascribed to the action of the caffein directly on the respiratory center, but rather to a secondary action through improvement of the circulation. In cases without dyspnea or in the cases in which the dyspnea had subsided, there was no effect, permanent or temporary, which could be clinically determined.

Effect on the Blood-Pressure.—The pressure was measured usually at 10 a. m. with a 10 cm. cuff, Riva-Rocci, by the auscultatory method. Chart 1 gives the pressure from day to day in each case, and in Chart 2 is given the composite curve of the fifteen cases. In twelve of the cases there was a marked drop in the pressure varying from 8 mm. in Case 12 to 50 mm. in Case 15. In Case 10, in which the endocardium was mainly at fault and there was little stasis, the blood-pressure remained at 135 mm. systolic. In Case 9 a pressure of 120 rose at first to 140 under caffein, but later dropped to 112 mm. In Case 3, in which the largest dosage was used, a case of luetic myocarditis confirmed at necropsy, the pressure rose under 100 grains of caffein daily from 172 systolic and 125 diastolic to 192 systolic and 150 diastolic. In three cases the drop was between 40 and 50 mm.; in three cases between 30 and 40; in three cases between 10 and 20, and in three cases below 10. The mean curve shows a slight drop the first day and a well-marked drop the second and third day with a gradual fall thereafter, the fall averaging about 2.5 mm. per day. That there is good clinical evidence that this drop in blood-pressure is due partly at least

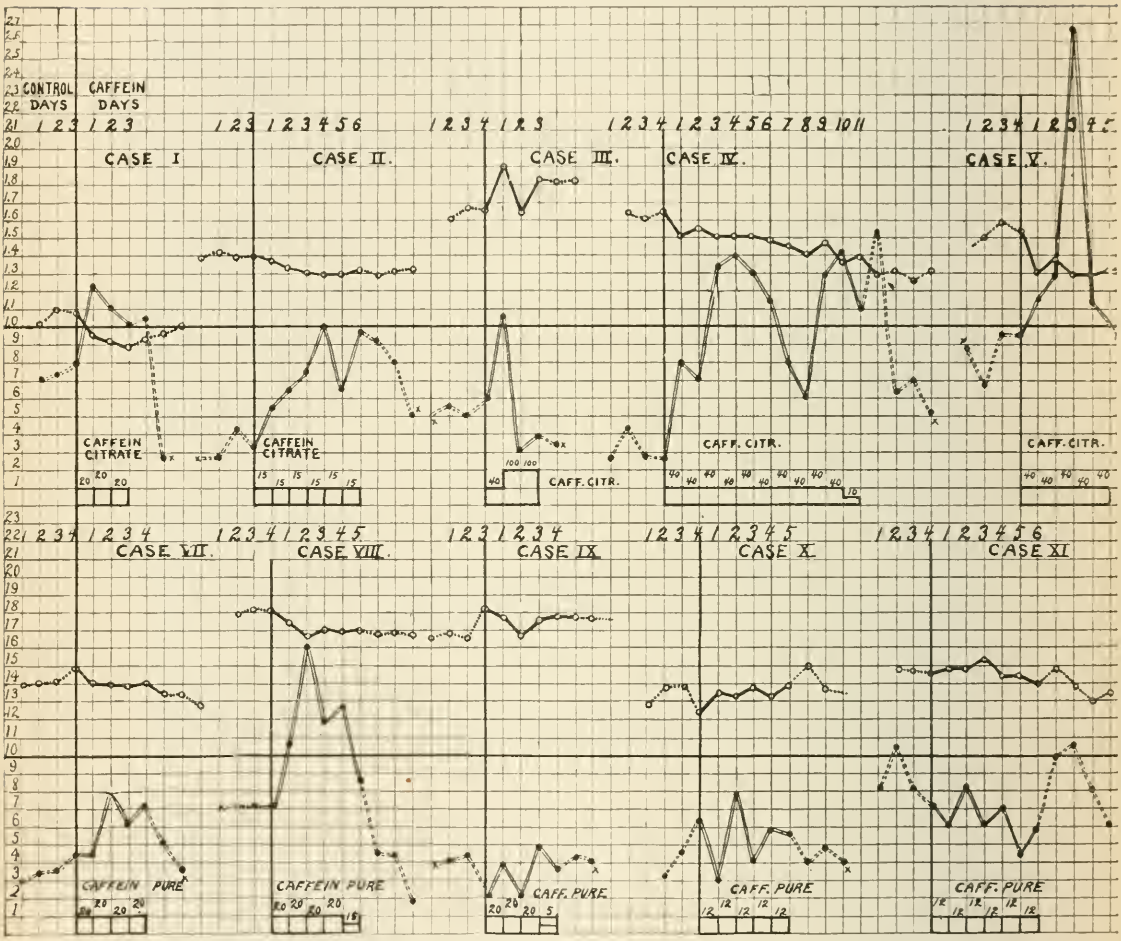
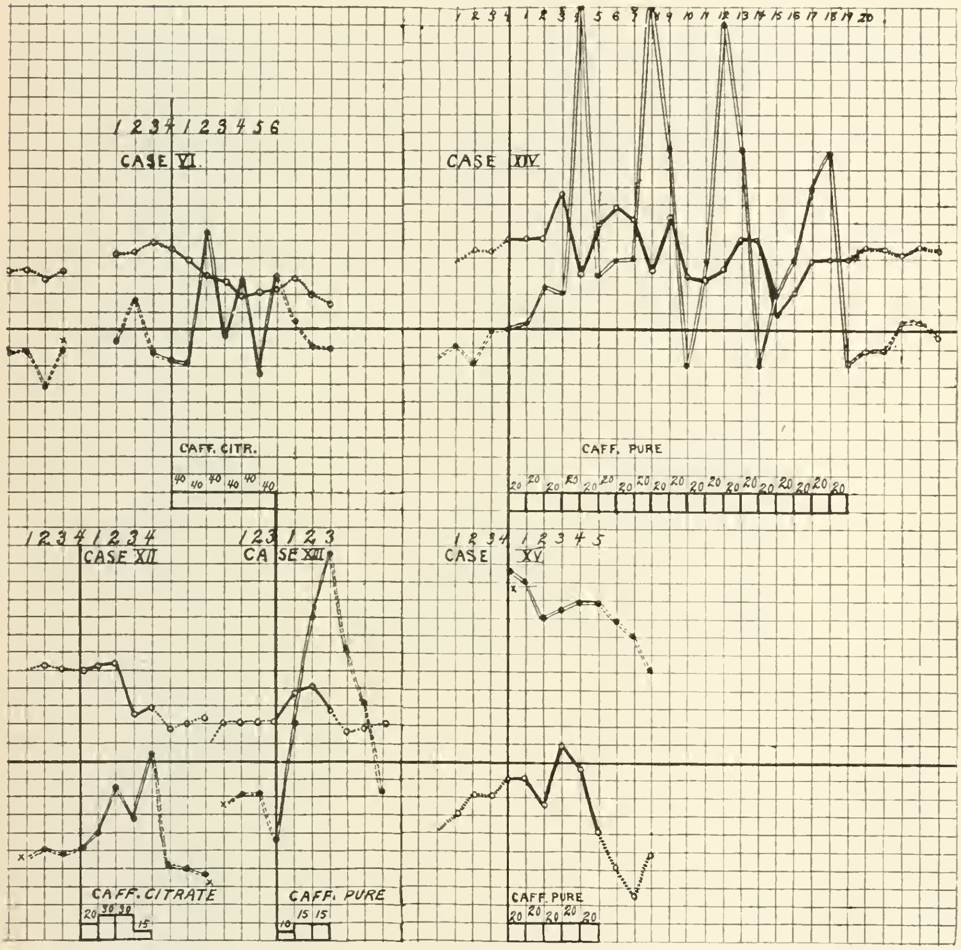


Chart 1. — Individual curve of blood-pressure (o --- o --- o — o — o) and diuretic coefficient and after the drug. The base line corresponds to a diuretic coefficient (output of urine divided by sure of 110 and diuretic coefficient of 1.1, etc. The dosage refers to grains per day of either citrated

to a peripheral dilatation of the vessels will be discussed later. From the charts then we may conclude that the final effect of caffein on the blood-pressure is to produce a depression, although in a few cases this may be preceded by a temporary rise on the first day. The fall is apt to continue after the administration is stopped.

Effect on Diuresis. — Chart 1 also serves to show the effect of caffein on the "diuretic coefficient." By the diuretic coefficient we mean the relation between the urine output and the fluid intake of the body. In the charts given here, for example, a diuretic coefficient of 0.6 means that the urine output equaled six-tenths or 60 per cent. of the fluid intake. So also a diuretic coefficient of 2.8 represents a urine excretion that is 280 per cent. of the intake. The individual cases are shown in Chart 1, while in Chart 2 the mean curve is shown. As plotted here it shows a prompt and sudden jump in the diuretic coefficient, a rise which progresses steadily to its maximum on the fourth day and then



(●═●═● ::::● ::::●) of 15 patients. Solid lines during caffein; broken lines before
income of fluid) of 1.0, and to a blood-pressure of 100 mm. Hg. 1.1 = blood-pres-
or pure caffein, as shown on the curve.

subsides. This return of the urine flow toward the normal is doubtless due to the smaller amount of disposable fluid, as the edema disappears. In all fifteen of the cases there was some rise on the second or third day, although in three of the cases it was not sufficient to be of any clinical importance. In two of these twelve cases the urine output equaled or excelled the fluid intake. It will be noted from the individual curves that while the ascent of the curve is not so marked on the third and fourth day, nevertheless the cases that respond most favorably to the drug show their greatest activity at that time. The less favorable cases show their activity on the first and second day and subside again. In two cases the stopping of the drug produced a secondary rise. In three of the four cases with their maximum on the fourth day the diuretic coefficient was 2.1, 2.7 and 2.8.

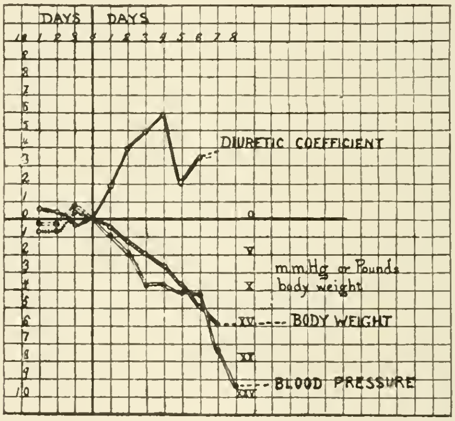


Chart 2.—Mean curves (compiled from the fifteen cases) of diuretic coefficient body, weight and blood-pressure. The base line corresponds to the level when caffein was started. Each square corresponds to a change of 0.1 in the diuretic coefficient, of 2.5 mm. of blood-pressure, and of 2.5 pounds of body weight.

When the mean curves of the blood-pressure and the diuretic coefficient are superimposed, the relation of these two factors seems at least suggestive, the diuresis varying inversely as the pressure. I would recall here the work of Loewi, who demonstrated that diuresis under caffein in the lower animals was due largely to a dilatation of the renal vessels. That there is also a general vasomotor relaxation has been demonstrated, although the cardiac action of caffein is usually regarded as sufficient to maintain or even elevate the blood-pressure. That caffein increases the tone of the blood-vessels is a popular therapeutic idea which animal experiment and clinical observation deny. Taking the cases singly, one finds that the day with the largest drop in the pressure there will also appear the largest urine output. And throughout, those cases which showed the greatest elimination of body fluid also showed the greatest general drop in blood-pressure. Of special interest was Case 14 in Chart 1. Here are shown very marked oscillations in the curve, the maximum diuretic coefficient appearing at four-day intervals, and associated with a marked drop in blood-pressure. A drop in the systolic pressure from 160 mm. to 130 mm. would be associated with a jump in the diuretic coefficient. This action was probably due to a spasmodic hypertonus, and the relief of the hypertonus could very well be absolutely independent of the caffein, although these blood-pressure changes did not appear until the caffein was begun. In the other cases, however, we did not see such marked action from the caffein on the blood-pressure. It can hardly be explained by the action of caffein on the vasomotor centers, because we find that after caffein the normal irritability is reduced, contrary to what we might expect from its other cerebral action. We feel, therefor, that the clinical diuretic action of caffein is the result of reduced pressure and vasodilatation.

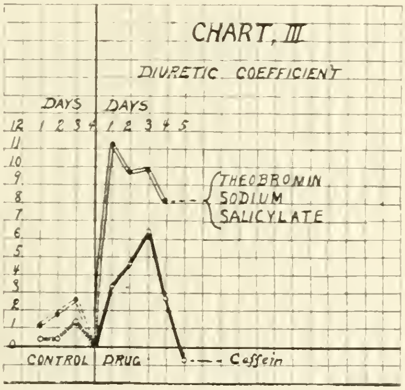


Chart 3.—Comparison of caffein and theobromin sodium salicylate. Mean curve of diuretic coefficient from five patients (3, 6, 7, 9, 13), on whom both drugs were used under similar conditions.

Effect on the Body Weight.—As is to be expected, the weight decreases with the loss of fluid by the diuresis, so that the curve of the body weight (Chart 2) will be seen to vary inversely as the curve of the diuretic coefficient. These individual curves could not be included with the blood-pressure and the diuretic coefficient without complicating the curves so that they would be unintelligible. Only the composite curve is shown here. The weight of the patient's stool was considered, and in these curves we can tell when the loss of weight is due to the loss of body fluid. The first day shows a small drop which continues steadily, the largest drop occurring in cases in which the action extended over six or eight days. Three cases, Nos. 6, 9 and 11, showed a small gain in weight up to 4 pounds with increased retention of body fluid, the other cases showing a loss varying from two to twenty pounds consistent with their urine output. Chart 2 shows the relation of the composite curves of the blood-pressure, diuretic coefficient and the body weight after caffein administration and with the four days' control.

Unpleasant Symptoms.—One of the most constant findings was the unpleasant symptoms from the drug. The caffein was given in capsules or powders by mouth, in 3 ounces of water, rather than hypodermatically, in order that its use might correspond to the ordinary clinical administration. These effects were mainly nervous and gastric. In thirteen of the fifteen cases there was discomfort shown from the drug. Eight patients were unable to sleep; seven complained of a vague uneasiness, a sense of restlessness; seven had nausea; five vomiting; four headache; two could not eat, and one besides being unable to eat or sleep insisted that the medicine gave him a pain in the side, the cause of which was not determined. The doses used were full doses, it is true, but no larger than necessary to secure results, and when they were reduced below the point where they gave nervous phenomena their cardiac value had disappeared.

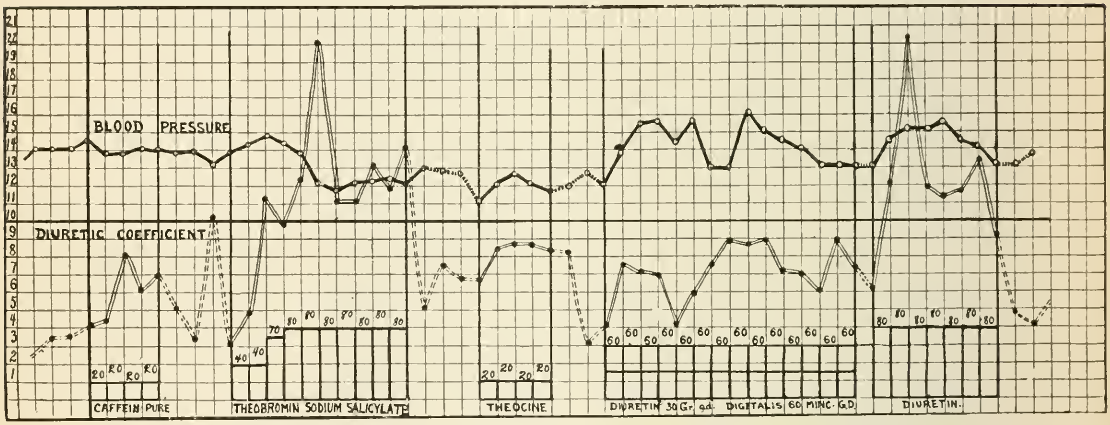


Chart 4.—Comparison of several drugs on same patient (Case 7). The upper curve is the blood-pressure; the lower, the diuretic coefficient. The doses are grains per day.

Clinical Efficiency.—Grouping the cases according to their clinical action, the drug has been credited with excellent results, moderate or none, according as it has relieved the subjective symptoms of cardiac origin, caused a diuresis or removed the objective signs. Of the fifteen cases the results were excellent in eight; moderate in four, and there was no result in three. In one of the cases where there was no result, the vomiting and loss of sleep even on small doses more than balanced any possible good that the drug could have done. All the cases with limited results showed the nervous signs, so the absorption of the drug was assured.

Comparison with Theobromin Sodium Salicylate.—In five of these cases a second admission to the hospital or a return of symptoms while they were still in the institution made it possible to try the comparative therapy of theobromin sodium salicylate under the same conditions and on the same heart on which the caffein action had been tested. Two charts are shown. One, Chart 3, represents two composite curves of these five cases. They show the mean curves of the caffein and theobromin sodium salicylate action on the diuretic coefficient, and are self-explanatory. The other, Chart 4, represents the relative effects of various drugs on the blood-pressure and diuresis in the same case, an old myocarditis. This chart is in accord with the other charts and our clinical experience in demonstrating that, while the action of caffein as a cardiac diuretic may be proved, still it is often of no clinical importance and is very much weaker than the other diuretics.

Our conclusions in regard to theobromin sodium salicylate are that its action on the urinary outflow is more prompt than that of caffein and the diuresis is larger. One can usually tell in twenty-four hours, in a given case, whether theobromin sodium salicylate will act or not; while with caffein the third or fourth day is more apt to show results. The theobromin sodium salicylate action is equally well sustained and usually at a higher level than the caffein. Where caffein has a depressing effect on the vasomotor system and lowers the blood-pressure, theobromin sodium salicylate first elevates the pressure and then later lowers it. The dosage of theobromin sodium salicylate we have found to play an important part in its action. As shown in Chart 4, a dosage of 30 grains daily, even though combined with digitalis, gave but little result; 40 grains daily gave a moderate diuresis, but a vigorous and prompt diuresis is the result of pushing the dose to 80 grains a day. In the chart this fact may be seen at two different points. This has been a frequent clinical experience, so that we regard 80 grains a day as a normal clinical dose. Patients will tolerate this dose easily, and this is an important practical factor in choosing a drug. Not only is the theobromin sodium salicylate action prompter, much stronger and equally well sustained, but we have found none of the gastric and nerve disturbances attendant on caffein. It is useless to give theobromin sodium salicylate in a given case and wait any length of time for its action to appear. In this group of cases with myocardial edema, experience with both drugs demonstrates the decided superiority of theobromin sodium salicylate over caffein. The relative value of the two drugs in arterial disease, cerebral and coronary sclerosis, is of course an entirely different problem.

Effective Dosage of Caffein.—In cases with edema, the diuretic effect of the drug may be safely taken as a sign of an improved mass movement of blood, which is the result we desire. From this point of view it is evident in this series that the small doses advocated by Romberg are not adequate. The best results were obtained in Cases 4, 5, 6, 8 and 14 with full doses. A good result was also obtained in Case 13 with a smaller dose, but even this dose, from 10 to 15 grains daily of the pure caffein, is much larger than that advocated by Romberg, who uses 2 grains for three doses. Inversely, in the cases with the smaller dose, Cases 2, 9 and 11, there was little clinical value from the drug. By administration by mouth the larger doses must be used to be efficient and almost always at the risk of unpleasant symptoms. The doses were often pushed to the limit of the patient's comfort, but there was no evidence that such doses produced an oliguria or a reversal of the vascular action. The hypodermic use of caffein in acute pulmonary edema and other acute heart conditions as a prompt cardiac stimulant will be taken up in another paper, in which I shall also present findings as to the nature of this caffein diuresis and the action of the drug on renal anasarca. In this series the hypodermic method was not employed, because even in hospital practice so many repeated injections have their disadvantages.

SUMMARY

In myocardial insufficiency with retained body fluid, caffein causes:

1. A moderate increase in the urine output with a proportional loss of body weight. This increase reaches its maximum on the fourth day.
2. A drop in both the systolic and diastolic blood-pressures which may stand in a causal relation to the diuretic coefficient, contrary to the usual teaching.
3. A slight (3.6 per cent.) temporary rise in the pulse-rate, but no permanent change in either the pulse or respiratory rate.
4. A moderate relief of the cardiac symptoms.
5. The constant appearance of distressing nervous and gastric symptoms.

The clinical diuretic action of caffein may be better performed by large doses of theobromin sodium salicylate without the unpleasant side effects.

This preliminary report is presented with a feeling of deep gratitude to Dr. C. F. Hoover and Dr. Torald Sollmann for their generosity with material and constant valuable suggestions in carrying on this work.

POSTANESTHETIC GLYCOSURIA AS INFLUENCED BY DIET, BODY TEMPERATURE AND PURITY OF THE ETHER*

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In 1904 one of us[1] reported the occurrence of glycosuria when meat-fed or fasting dogs were subjected to ether anesthesia for varying periods of time. In the case of the meat-fed animals the percentage of dextrose in the urine fraction first voided after anesthesia ranged from 1.32 per cent. to 6.94 per cent. In the case of the fasting dogs the percentage ranged from a negative test to 0.52 per cent.[2] In 1905 Seelig[3] confirmed these findings. At this time (1904-6) all ethers used for purposes of anesthesia contained aldehyd and other impurities. Recently Prof. Charles Baskerville has succeeded in purifying ethers,[4] and it was he who suggested that we investigate the relationship of this purified or so-called "dehydrated" ether to postanesthetic glycosuria.[5]

Two other phases of the glycosuria question were incorporated with the study of the glycosuria-producing properties of the "dehydrated" ether, that is, the influence of diet and of the lowering of body temperature on the production of post-anesthetic glycosuria. We shall first discuss the body temperature factor.

I.—RELATIONSHIP OF BODY TEMPERATURE TO GLYCOSURIA

Araki[6] was one of the first investigators to demonstrate that a pronounced lowering of the body temperature of an animal by *external* means caused the appearance of sugar in the urine of that animal. He experimented with rabbits and dogs, using baths of ice-water and of snow to secure low body temperatures. Of five experiments on rabbits three were positive, the urine containing from 0.75 per cent. to 2.5 per

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* From the Laboratories of Physiological-Chemistry of Jefferson Medical College and the University of Illinois.

1. Hawk, P. B.: Proceedings of American Physiological Society, Am. Jour. Physiol., 1904, x, 37.

2. Hawk, P. B.: Postanesthetic Glycosuria, The Archives Int. Med., 1911, viii, 39.

3. Seelig: Arch. f. exper. Path. u. Pharm., 1905, lii, 481, also 1906, liv, 206.

4. Baskerville and Hamor: Jour. Indust. and Engin. Chem., 1911, iii, 1. See also Gwathmey and Baskerville's Anesthesia, 1914, Appleton.

5. We wish to thank Professor Baskerville and Squibb & Sons for their kindness in furnishing the ether used in these experiments.

6. Araki: Ztschr. f. physiol. Chem., 1891-2, xvi, 453.

cent. of sugar. Two tests on dogs showed urinary sugar percentages of 1 per cent. and 1.5 per cent., respectively. The rabbits were given snow baths ranging in length from one hour forty minutes to two hours twenty minutes. The body temperature was lowered to 27 C. or lower. One of the dogs which was subjected to a snow bath for three hours twenty minutes showed a drop in body temperature to 23 C., whereas the other dog, which was placed in an ice-water bath for fifty minutes showed a minimum temperature of 26 C. The former animal had 1.5 per cent. sugar in its urine; the latter animal voided a urine containing 1 per cent. of sugar. The appearance of the sugar was interpreted as due to incomplete oxidation.

Several years later Lüthje[7] and Embden, Lüthje and Liefman[8] demonstrated that the quantity of sugar present in the urine of dogs subjected to pancreatic diabetes was dependent to a degree on the temperature of the dog's environment. For example, one dog kept in a cold room excreted 47 gm. of sugar, whereas the excretion of this same animal was only 4 gm. when the dog was maintained in a warm environment. Lüthje interpreted his results as indicating that under the influence of a cold environment more sugar was produced in an attempt to maintain body temperature by its oxidation.

Brasch[9] and Minkowski[10] in turn strongly criticized Lüthje's findings and conclusions. Brasch experimented with dogs subjected to phlorhizin diabetes and found that the lowering of body temperature had no influence on the sugar excretion. Minkowski and Allard, working with dogs after pancreas extirpation, also demonstrated that a lowered body temperature had no effect on the sugar output. The findings of Lüthje are interpreted by Brasch and Minkowski as being due to the fact that the pancreas was only partly extirpated. This being the case, under the influence of the lowered temperature some glycogen was changed into sugar and passed into the circulation. Mohr[11] confirmed the findings of Brasch and Minkowski in that he was unable to influence the sugar excretion in pancreatic diabetes by lowering the temperature of the animal's environment.

Glaessner[12] calls attention to observations which indicate that a pronounced cooling of the human body by external means, such as cold water, may produce a transient glycosuria. In nine cases of reduced temperature sugar was present in only four instances in the first fractions of urine subsequently passed.

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[7] Lüthje: Verhandl. d. Cong. f. inn. Med., 1905, p. 268.

[8] Embden, Lüthje and Liefman: Beitr. z. chem. Physiol. u. Path. (Hofmeister's), 1907, x, 265.

[9] Brasch: München. med. Wchnschr., 1906, vii, 805.

[10] Minkowski: Verhandl. d. Cong. f. inn. Med., 1907, p. 272.

[11] Mohr: Ztschr. f. exper. Path., 1907, iv, 910.

[12] Glaessner: Wien. klin. Wchnschr., 1906, xix, 920.

An interesting experiment on the influence of cold on the sugar excretion in phlorhizin glycosuria has been made by Lusk.[13] In one experiment an 18 kg. dog after fasting for three days and then being permitted to shiver "in the cold of a north wind" for six hours showed an increase of 8.47 gm. in the dextrose output. Exposure to a cold environment for seven and one-half hours on the following day caused an increase of only 6 gm. in the sugar excretion. In the case of another dog (9 kg.) which had fasted one and one-half days, exposure to cold for six hours produced an increase of 4.44 gm. in the dextrose output, whereas a second exposure of six and three-quarter hours on the following day caused no increase in the sugar excretion.

Lusk concludes that the reduction of body temperature by external means "has no effect on sugar production in the fasting organism, except in so far as body glycogen may be converted into dextrose.

Grube[14] has more recently reported data from a series of observations which indicate that no glycosuria follows the anesthetization of meat-fed dogs by means of ether, provided care is taken to maintain the body temperature approximately normal during the anesthesia period. He reports five experiments on dogs ranging in weight from 3.5 kg. to 8.7 kg. in which sugar excretions ranging from a trace to 7.16 per cent. were obtained. The lowering of body temperature ranged from 2.6 to 4.6 C. Grube then noted that no glycosuria resulted in one experiment in which for some unexplained reason the body temperature of the dog under examination was not lowered under the influence of the ether. Following this he made two or three tests in which the body of the animal was maintained at or above the normal temperature during the period of anesthesia. The temperature was regulated by wrapping the dog in cloths and placing the animal on a specially prepared water-bath. In no case was there any sign of glycosuria in an animal whose body temperature had been maintained near the normal by artificial means.

OUR EXPERIMENTS

Dogs were used as subjects. The animals were fed a diet of meat, lard, graham crackers, bone-ash and water during Experiments 1 to 14 (see Table 1) whereas in Experiments 15 to 24 the animals were fed a "carbohydrate-free" diet (see Table 2). Urine collections were made in fractions as voided for thirty-six hours after each anesthetization. The urines were examined by polariscope before and after fermentation.

Three methods of anesthetization were employed, the period of anesthesia being two hours in each instance. The "cone method" consisted in inserting the dog's nose into a perforated tin cone which contained in its bottom a wad of cotton saturated with ether. The so-called "improved method," devised by us, was accomplished by the use of a special apparatus. This apparatus furnishes the animal with warm ether vapor which is saturated with water vapor and mixed with air. This procedure reduces the loss of heat from the body by warming the vapors which are breathed, and by insuring the saturation of the vapors with water causes a reduction in the heat lost by evaporation from the respiratory organs. The current of compressed air insures an abundance of oxygen. For purposes of experiment cold ether vapor was secured by replacing the warm water of the bath by a freezing mixture of salt and ice.

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[13] Lusk: Am. Jour. Physiol., 1908, xxii, 163.

[14] Grube: Arch. f. d. ges. Physiol., 1911, cxxxviii, 601.

In order to permit the maintenance of normal body temperature during the progress of anesthesia a so-called "thermos-box" was devised. This box was fitted with sixteen sockets for electric light bulbs, each light being capable of individual manipulation. By this means the body temperature of the animal was easily maintained practically constant even during long periods of anesthesia. Losses by radiation and conduction from the surface of the animal were compensated for by the heat furnished by the "thermos-box."

TABLE 1.—Anesthetization of Dogs Which Were Fed on a Mixfd Diet *

Experim't No.	Dog No.	Date	Ether Used	Method of Anesthesia	Range of Body Temperature
1	13	11/19	Dehydrated	I. M. cold †	39.0-33.0
2	12	11/21	Dehydrated	I. M. cold	39.0-35.5
3	13	11/23	Dehydrated	I. M. warm‡	Constant
4	14	11/24	Dehydrated	I. M. warm	Constant
5	12	11/25	Dehydrated	I. M. warm	Constant
6	14	11/28	Dehydrated	I. M. cold	Constant
7	13	11/27	Common	I. M. warm	Constant
8	12	11/29	Common	I. M. warm	Constant
9	13	11/30	Common	I. M. cold	39.2-34.8
10	12	12/ 2	Common	I. M. cold	39.6-36.2
11	13	12/ 3	Common	Cone	39.3-31.6
12	12	12/ 4	Common	Cone	38.8-29.0
13	13	12/ 5	Common	Cone	39.3-32.1
14	12	12/ 6	Common	Cone	39.8-28.7

* Fehling and fermentation tests on urine negative throughout.
† I. M. cold signifies improved method using cold ether vapor.
‡ I. M. warm signifies improved method using warm ether vapor.

If the data from Experiments 1, 2 and 9 to 14, listed in Column 6 of Table 1, be examined, it will be observed that temperature depressions ranging from 3.4 C. to 11.1 C., were obtained in the course of eight experiments. Even with such pronounced lowerings of body temperature, however, the urine never contained a trace of sugar. It is evident, therefore, that so far as our experimental animals are concerned, the lowering of their body temperature by means of ether inhalation did not produce glycosuria.

The body temperature underwent the most pronounced reduction when the anesthetic was given by the "cone method." This was due principally to the extreme dryness of the inhaled ether vapor.

II.—THE FACTOR OF DIET IN POSTANESTHETIC GLYCOSURIA

If the data contained in the final columns of Tables 1 and 2 be examined it will be observed that every anesthetization listed in Table 2 was accompanied by glycosuria, whereas sugar was never present in the urine after the periods of anesthesia listed in Table 1. The only variable factor in the two sets of anesthetizations was the diet. The same animals were used in each series, the same kinds of ether were employed, the anesthetic was given by the same methods and for the same length of time. Previous to the administration of the ether in Experiments 1 to 14, however, the dogs were fed the usual mixed diet, whereas previous to Experiments 15 to 24 they were fed a carbohydrate-free diet.

TABLE 2.—Anesthetization of Dogs Which Were Fed on a Carbohydrate-Free Diet

Experim't No.	Dog No.	Date	Ether Used	Method of Anesthesia	Range of Body Temperature	Dextrose in Urine gm.
15	13	12/14	Dehydrated	I. M. cold	39.0-36.7	0.53
16	12	12/15	Dehydrated	I. M. warm	Constant	0.39
17	13	12/16	Dehydrated	I. M. warm	Constant	0.19
19	13	12/20	Dehydrated	I. M. warm	Constant	0.45
20	15	12/21	Dehydrated	I. M. warm	Constant	0.53
21	14	12/22	Dehydrated	I. M. warm	Constant	0.65
23	12	12/13	Common	Cone	39.7-35.0	1.44
24	14	12/17	Common	I. M. cold	39.0-32.9	0.38

At the beginning of our tests we were exceedingly surprised at our inability to detect any trace of sugar in the urine of our dogs even after the animals had been under ether for a period of two hours. After conducting fourteen experiments on four different animals, using two different ethers and administering the ether by three different methods and failing in any case to produce any sign of glycosuria we decided to note the influence of the removal of carbohydrate from the diet. The data from the series of tests on the carbohydrate-free diet are given in Table 2. An examination of the final column of that table will show that in every instance the anesthetization of an animal which had been fed a carbohydrate-free diet, resulted in the production of glycosuria. Further work is contemplated in order to determine the minimum carbohydrate ingestion which will prevent the occurrence of postanesthetic glycosuria. The data may have a clinical application.

In order to determine whether lack of oxygen during the ether inhalation could of itself cause glycosuria several experiments were made by various methods of anesthesia. A mixed diet was used in every instance. By the so-called "improved method" the maximum amount of air was mixed with the ether vapor before inhalation, whereas in the "cone method" the minimum amount of air was allowed to be breathed with the ether vapor. There was no glycosuria in any case, indicating that the lack of oxygen in these cases was not sufficient to cause glycosuria.

III.—"DEHYDRATED" VERSUS COMMON ETHER

The dehydrated ether employed was specially prepared for us by Professor Baskerville, whereas the common ether was the regulation Squibb ether. The opinion having been expressed that the postanesthesia glycosuria and other undesirable features accompanying or following ether inhalation were due not to the ether per se, but to some impurity in the ether, we were very glad of the opportunity of making a series of tests in an attempt to elucidate this problem. The particular thing in view in this instance was to determine the relationship of these two types of ether to the ether glycosuria.

In Table 1 are listed six experiments with "dehydrated" ether and eight experiments with common ether. *In no case was glycosuria observed.* The animals were fed a mixed diet. In Table 2 are listed six experiments with "dehydrated" ether and two with common ether. *Glycosuria was observed in each instance.* The dogs were fed a carbohydrate-free diet.

The experiments force us to the conclusion that the glycosuria following ether inhalation is not due to impurities present in the ether, since the purest ether obtainable yields just as pronounced glycosuria as does the regulation Squibb ether used for anesthesia purposes. Our experiments indicate that the occurrence or non-occurrence of glycosuria hinges on the character of the diet, the ingestion of a carbohydrate-free diet yielding glycosuria, whereas the ingestion of a mixed diet is unaccompanied by glycosuria.

CONCLUSIONS

1. The reduction of body temperature during ether anesthesia does not of itself cause glycosuria.

2. The reduction to a minimum of the oxygen content of the ether vapor inhaled does not cause glycosuria.

3. The response of the animal body to "dehydrated" ether (that is, ether from which aldehyd and other impurities have been removed) in so far as glycosuria is concerned, is no different from the response to the ether ordinarily used (Squibb) as an anesthetic.

4. The inhalation of ether administered by the cone method causes a more pronounced reduction in body temperature than does the inha-

lation of the same kind of ether for a similar period when administered by the so-called improved method. The dryness of the ether vapor in the cone method is the contributing factor.

5. The inhalation of either "dehydrated" or common ether for a period of two hours by dogs fed a carbohydrate-free diet was in every instance accompanied by glycosuria. When the same animals were fed a mixed diet glycosuria was not in evidence.

6. There was no evidence of "emotional" glycosuria, the factor regulating the occurrence or non-occurrence of glycosuria in every instance being a dietary one.

We wish to express our thanks to Dr. Paul E. Howe for assistance rendered.

THE COMPLEMENT-DEVIATION TEST WITH BESREDKA'S TUBERCULIN AND THE OCCURRENCE OF TUBERCULOSIS AMONG SYPHILITICS AS DIAGNOSED BY THIS TEST *

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Although tuberculosis is one of the most common of infectious diseases, it is one of the most difficult to diagnose in its early stages. Very many cases progress to spontaneous recovery without ever giving the slightest possibility of diagnosing the process, and only the statistics of post-mortem examinations show how frequently healed and unhealed undiagnosed tuberculous conditions have existed. It is true that by means of the von Pirquet test many cases in which tuberculosis has existed some time during the patient's life can be diagnosed, but the extreme sensitiveness of this test makes it impossible to differentiate cases in the active stages of the disease from th which the process of the disease was arrested. For this reason results of the von Pirquet test, though valuable for the exclusion of tuberculosis, are of little value for the diagnosis of the disease, especially in adults. Many and continuous attempts have been made to apply the methods of diagnosis of other infectious diseases to that of tuberculosis, but all of these attempts have so far met only limited success.

In the present communication I shall report on a method of complement deviation in tuberculosis which seems to answer the requirements both of early diagnosis and of differentiation of active tuberculosis from that in which the pathologic condition has apparently been arrested. The main reason why complement deviation in tuberculosis has failed so far, in spite of the many attempts, seems to have been the lack of a suitable antigen. This gap seems at last to have been filled through the discovery by Besredka of a new method of cultivating tubercle bacilli.[1] These organisms, grown on a medium consisting of a suitable mixture of bouillon, egg-yolk and egg-white, show biologic properties distinctly different from those observed heretofore, one of the most striking being the rapidity of their growth,

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* Submitted for publication June 17, 1914.

* From the Pathological and Research Laboratories of the Western Pennsylvania Hospital.

[1] Besredka, A.: Compt. Rend. Acad. d. Sc., 1913, clvi, 1633.

which equals that of streptococcus, for instance. This culture, heated and deprived of its cellular elements by filtration, proved, in preliminary experiments of Besredka and Manoukhine,[2] to be very efficient both as tuberculin and as antigen for the complement-deviation test.

Professor Besredka's kindness in sending me his antigen, enabled me to substantiate fully his preliminary findings as to the efficiency of his antigen for early diagnosis of tuberculosis, both in animals and in man. Experimental animals (rabbits and guinea-pigs) infected with tuberculosis gave a positive reaction by this method often as early as from seven to ten days after the infection; on the other hand, among a group of six hundred cases examined up to April by this method, I found in many instances this reaction present before any other definite symptoms of the disease were noted.

The technic used in the examination was the following:

Antihuman hemolytic serum was used, with a very small amount of complement, two units, carefully controlled by titration, two units of antihuman amboceptor and 0.04 c.c. of the antigen (tuberculin of Besredka), a quantity which was found to contain more than five antigenic units, being at the same time at least five times smaller than the dose fixing complement by itself. In about the first fifty cases both active and inactive serum was used, showing no difference in the results except for a few specimens which, as they came several days after collection, had developed anticomplementary properties. As, on the one hand, in these cases, heating of the serums was essential for the destruction of the anticomplementary properties, and, on the other hand, it was important to treat all serums alike to obtain comparable results, a uniform technic was adopted, namely, to heat all the serums for half an hour at from 54 c.c. to 56 c.c., using 0.1 c.c. of the serum.[3]

As the antigen used in these experiments is represented by a filtrate of cultures of tubercle bacilli grown on mediums consisting of bouillon, egg-yolk and egg-white, the possibility of non-specific lipotropic fixation had to be excluded. As a control, therefore, a test with a pure lipoid antigen was made in each case. From the very beginning unexpected results were obtained, namely, a great number of the serums giving positive tuberculous reaction by this method, gave also positive Wassermann reaction. Therefore, though Besredka in placing his antigen at my disposal suggested to me to study in the main, if possible, the occurrence of the reaction on a series of patients at different stages of their disease, these findings called my attention for the time being to the question of the specificity of this reaction, and I shall in this paper report the results of my findings along these lines.

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[2] Besredka and Manoukhine: Compt. Rend. Soc. Biol., 1914, lxxvi, 180.

[3] Dovidowitch reports (Deutsch. med. Wchnschr., 1914, No. 1, p. 21) that tuberculous serums giving positive reaction with the tuberculin used by him as antigen, failed to fix the complement after being heated for half an hour at 56 C. (132.8 F.), from which he concluded that tuberculous antibody is markedly thermolabile. I found (1909) that fresh serum at times contains substances possessing the power of fixing the complement in presence of antigen containing protein (proteotropic fixation) and that these substances are destroyed through incubation at 56 C. for half an hour. The statement of Dovidowitch suggests, therefore, that the fixation with his tuberculin was of the nature of a proteotropic reaction, and hence his findings. Using Besredka's antigen, serums heated at 56 C. for half an hour continued to fix complement.

My work was along two distinct lines. In one series of experiments I tried to prove the independent coexistence of the two antibodies in the same specimen of serum directly, in another series I tried to experiment on patients or animals, as the case might be, and in this way follow the fate of the corresponding reactions.

The first explanation for the coincidence of the positive tuberculous and Wassermann reactions naturally was that serums which had a high lipotropic coefficient fixed also with tuberculous antigen on account of the lipoid it contains. I therefore decided to study the quantitative relationship between the fixing power of the same serums in presence of each of the two antigens. The comparison was made in the following manner:

Each serum was diluted with salt solution, and for each case a series of tubes was set up, in which decreasing amounts of diluted serum were used, the rest of the reagents remaining as above; in this way the minimal dose of serum capable of fixing two units of complement in presence of five units of tuberculous antigen was determined for each specimen of the serum. At the same time, in a parallel series, the same serums were titrated against syphilitic antigen in the same manner, except that instead of the five units of tuberculous antigen five units of pure lipoid antigen were used. Table 1 represents the comparative results of the examination of a few specimens selected out of our series.

Table 1 shows that although a serum may have a high antibody-index as tested against one antigen, it may at the same time show but a very low index against the second antigen, or indeed an index zero, as in Cases 182 or 219 in Table 1, in which Serum 182, for instance, showing 70 units of tuberculous antibody for each 0.1 c.c. showed no antibody at all when tested against syphilitic antigen, proving that there is no relationship between the degree of fixation displayed by the given serum against each of the two antigens. The absence of any regularity in this relationship is especially clearly shown in Table 2. Out of the series of cases I selected 20 giving the same antibody index, as tested against Besredka's tuberculin, as can be seen from Table 2.

These twenty cases gave most varied lipotropic indices; namely, two cases showed quite a high antibody content of 500 units; three cases of 300; two of 150; two of 50; two of 30; one of 10 units and eight cases contained no lipotropic antibody at all. Similarly in other twenty cases with the same lipotropic index, varying tuberculous indices were found, thus illustrating quite conclusively that the rela-

TABLE 1.—ANTIBODY INDEX WITH VARIOUS ANTIGENS

	Case No.	Amount of Serum Used for Titration (in c.c.)																			Minimum fixing dose in c.c. (unit of antibody)	No. antibody units per 0.1 c.c. of serum
		0.1	0.07	0.05	0.03	0.02	0.015	0.01	0.007	0.005	0.003	0.002	0.0015	0.001	0.0007	0.0005	0.0003	0.0002	0.00015	0.0001		
Examined with 0.0001 c.c. of pure lipoid antigen and 0.03 c.c. of complement.	116	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	MchH	CH	....	....	....	....	....	....	0.002	50
	124	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	....	....	....	0.003	30
	134	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	MchH	CH	....	....	....	....	NoH	FCH	CH	0.0002	300
	154	CH	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	......	0
	160	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	SlH	CH	....	....	....	....	....	....	....	....	....	0.01	10
	182	CH	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	......	0
	206	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	CH	CH	....	0.0003	300
	219	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	CH	CH	0.0002	500
Examined with 0.04 c.c. of tuberculin-Besredka and 0.03 c.c. of complement.	116	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	trH	CH	....	....	....	....	....	....	....	....	0.004	25
	124	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	trH	CH	....	....	....	....	....	....	....	0.003	30
	134	NoH	NoH	NoH	NoH	NoH	NoH	NoH	MchH	CH	....	....	....	....	....	....	....	....	....	....	0.01	10
	154	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	SlH	CH	....	....	0.0005	200
	160	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	FCH	CH	....	....	0.0005	200
	182	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	SlH	CH	....	....	....	....	....	0.0015	70
	206	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	MchH	CH	....	0.0003	300
	219	CH	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	......	0

NoH = No hemolysis or complete fixation (95 to 100 per cent.).
trH = Traces of hemolysis or incomplete fixation (80 to 90 per cent.).
SlH = Slight hemolysis or slight fixation (60 to 70 per cent.).
MchH = Much hemolysis or weak fixation (30 to 50 per cent.).
FCH = Fairly complete hemolysis or very weak fixation (15 to 30 per cent.).
CH = Complete hemolysis or no fixation (less than 15 per cent.).

The numbers in the last column to the right on this table represent the number of antibody units in each 0.1 c.c. of the serum (antibody index). The next column to the left shows the smallest amount of the serum necessary to fix the complement, in other words, the absolute quantity of serum containing one antibody unit for the given serum. By dividing 0.1 c.c. by this number, one can calculate the number of antibody units per 0.1 c.c. of each serum, or its antibody index.

tion between the two antibodies in the same serums does not show any tendency to conform to any law.

In order to decide further whether the two antibodies coexist in the same serum independently, I tried to exhaust one of the presumably coexisting antibodies in the serum by repeated incubation with one of the antigens and subsequently attempted to see whether or not in the process of exhaustion the antibody fixing the complement with the other antigen was removed. The technic of the experiment is the following:

A series of tubes is prepared containing all the ingredients as for routine examination, namely, 0.1 c.c. of heated serum known to give positive reaction with both antigens, 5 fixing units of antigen, plus 2 units of complement, plus 2 units of amboceptor, and salt solution to complete to 1 c.c. The tubes are incubated in water-bath at 37 C. for half an hour. At the end of this time, one tube is taken out from the series and 0.1 c.c. of 10 per cent. washed human blood-corpuscles added, the remaining tubes being kept at room temperature.

TABLE 2.—Showing Variation in Degree of Fixation with Different Antigens

	Tuberculosis Reaction	Wassermann Reaction						
No. of units of antibody..	500	0	10	30	50	150	300	500
No. of cases.............	20	8	1	2	2	2	3	2
	Wassermann Reaction	Tuberculosis Reaction						
No. of units of antibody..	500	0	15	50	100	300	500	More than 500
No. of cases.............	20	6	1	3	3	2	3	2

At the end of half an hour, when in the tube to which the blood-corpuscles were added no hemolysis is observed, all the remaining tubes of the series receive 2 additional units of complement, and 5 additional units of antigen, and are incubated at 37 C. for half an hour. Again one tube is taken out and 0.1 c.c. of 10 per cent. washed human blood-corpuscles added and the tube incubated for half an hour at 37 C., the rest of the tubes remaining at room temperature. If still no hemolysis in the separate tube is observed, the process is repeated, that is, 2 units of complement and 5 units of antigen are added to each tube of the series, one tube of the series tested, etc., until finally all the antibody from the serum is exhausted and the tube in which the test is made shows complete hemolysis. At this point one of the remaining tubes of the series receives 2 additional units of complement, and 5 additional units of antigen, and another tube receives, as before, 2 units of complement and 5 units of the first antigen as a control. After incubation in a water-bath at 37 C. for half an hour, 0.1 c.c. of 10 per cent. human blood-corpuscles are added; and, whereas, in the tube to which the second antigen was added there was no hemolysis, the other shows complete hemolysis, proving that the first antibody is completely exhausted without exhaustion of the second. The technic was slightly simplified in the later experiments and is shown in its simpler form in Table 3. Part "A" of this table should be noted as one hour ahead of Part "B" and in this way the results of "A" show when the exhaustion in "B" should stop.

The experiments shown in Table 3 were also reversed, namely, serum was exhausted of its tuberculous antibody and subsequently the presence of syphilitic antibody was determined. As can be seen from Table 3, the proper controls were carried to show that the fixation after the exhaustion of one antibody and addition of the second antigen was not due to a summing up of fixation by different ingredients, as Serum 280 was tested in absolutely the same manner as Serum 297, yet the addition of tuberculin after the exhaustion of syphilitic antibody in the first case did not cause any fixation, and the fixation in Case 297 therefore should be attributed to the specific antibody present in this serum. The same is true for the two other controls, 285 and 315, treated in a similar way, of which only one, namely 285, fixed complement with the tuberculin on account of the specific antibody it contained.

Although the serologic studies above reported and especially the exhaustion experiment demonstrate quite convincingly that when present in the same serums, the Wassermann reaction and the tuberculin reaction may be absolutely independent of each other, yet, in practice, the coexistence of the two antibodies would have to be proved in each case, as long as the antigen used in the reaction contains lipoid. It was for this reason that I undertook to free the tuberculin of Besredka of its lipoid, in order to eliminate the very possibility of non-specific reaction.[4] The antigen for this purpose was shaken up with ether, chloroform, benzol (benzene), and petroleum-ether in a separating funnel, and, subsequently, the solvent carrying the lipoid was separated from the protein part of the antigen. The latter part alone was then used as antigen for the complement-deviation test. The detailed report of these experiments will be published later in the collaboration with Dr. Rockman, who is comparing the antigenic value of this purified antigen with the original preparation in a number of cases. I may say, however, anticipating this report, that it follows from the 200 examinations up to date, that the value of the antigen was not in the slightest degree diminished by the extraction of the lipoid. As to the lipoid extracted from Besredka's tuberculin, as I expected, it can cause fixation of complement with syphilitic serums, if used in adequate amount: when a large amount of this lipoid, extracted from tuberculin, was collected, the ethereal extract evaporated to dryness, and a very concentrated emulsion in physiologic salt solution (0.03 per cent.) was prepared, it showed undoubted antigenic properties, as would lipoids extracted from any source. It was found, however, that the quantity of this lipoid present in the dose of tuberculin (0.04

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[4] Bronfenbrenner, J.: Proc. Soc. Exper. Biol. and Med., 1914, xi, 92.

c.c.) used as antigen in the test for diagnosis of tuberculosis, is entirely too small to cause lipotropic reaction, as is evident from the following experiment:

Ethereal extract of the lipoid from 10 c.c. of tuberculin was separated from the aqueous part by means of a separating funnel, concentrated to dryness, taken up with about 0.5 c.c. of ether, and salt solution added to bring the volume back to 10 c.c. In this way the lipoid was brought back to its original concentration in the tuberculin. The resulting emulsion was used as antigen for a Wassermann reaction in the dose (0.04 c.c.) in which the whole tuberculin was used as antigen (using serums known to give a strong Wassermann reaction when tested with 5 units of regular antigen).

TABLE 3.—Results of Experiment to Determine—

A. M.	Number of Exhaustions	Serum 297 0.05 c.c.					
		W (200)			Tb (200)		
9:00	1	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd
9:30	2	Amb Corp	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd
10:00	3	NoH	Amb Corp	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd
10:30	4	....	NoH	Amb Corp	Cpl Lpd	Cpl Lpd	Cpl Lpd
11:00	5	....	....	NoH	Amb Corp	Cpl Lpd	Cpl Lpd
11:30	6	....	....	....	CH	....	....
12:00	..	....	....	....	....	....	....
12:30	..	....	....	....	....	....	....

The number in parenthesis after the sign W and Tb means the titer of the serum with
The sign W(0) therefore means Wassermann negative and Tb(0) tuberculous reaction
(Cpl) it was throughout this experiment 0..03 c.c.; in case of lipoid antigen (Lpd) it
case of amboceptor (Amb) and human corpuscles (Corp) the sensitized corpuscles
manner, that each dose of each of the ingredients was represented in 0.05 c.c. volume. The

As can be seen from Table 4, three serums were selected for this experiment, each of them of a high lipotropic index. As to the fixation with Besredka's tuberculin, these serums showed respectively the titers 300, 10 and 0. Now if the fixation with Besredka's tuberculin in the case of Serums 206 and 134 was due to the presence of lipoid in the tuberculin, the emulsion of the lipoid taken in the amount in which it occurs in the tuberculin should, on the one hand, have fixed the complement with these serums; on the other hand, also Serum 219, showing the property to fix the complement in presence of the lipoid to the same degree (see section "A" Table 4), should have also given a positive reaction with tuberculin (containing lipoid) as well as with the emulsion of pure lipoid, extracted from it. As section "C" in Table 4 shows, no fixation whatsoever took place with any of these serums, however, when the lipoid extracted from the tuberculin was used in the quantity in which it occurs in the tuberculin. Only when the dose of this emulsion of lipoid was increased ten times (see section "D" of Table 4) it began to fix the complement in presence of the largest amounts of the serums. But even there the fixation of the complement is evidently due, not to the specific combination between lipoid serum and complement, but to a summing up of partial complement fixations caused by the large doses of serum as well as by the lipoid emulsion alone,[5] as in case of specific fixation, inhibition of

—WHETHER TWO ANTIBODIES COEXIST IN THE SAME SERUM

Number of of Exhaustions	Serum 297 0.05 c.c.		Serum 280 0.05 c.c.		Serum 285 0.05 c.c.		Serum 315 0.05 c.c.		A. M.
	W (200)	Tb (200)	W (200)	Tb (0)	W (0)	Tb (70)	W (0)	Tb (0)	
	....	....	....	....	....	....	....	....	9:00
	....	....	....	....	....	....	....	....	9:30
1	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	10:00
2	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	10:30
3	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	Cpl Lpd	11:00
4	Cpl Lpd	Cpl Tbc	Cpl Lpd	Cpl Tbc	Cpl Lpd	Cpl Tbc	Cpl Lpd	Cpl Tbc	11:30
	Amb Corp	Amb Corp	Amb Corp	Amb Corp	Amb Corp	Amb Corp	Amb Corp	Amb Corp	12:00
	CH	NoH	CH	CH	CH	NoH	CH	CH	12:30

respective antigens; W = Wassermann or lipoid antigen and Tb = tuberculous antigen.
negative. The dose of reagents was not indicated on the table. In case of complement
was throughout 0.0001 c.c.; in case of tuberculin (Tbc) it was throughout 0.04 c.c.; in
carrying amboceptor were used. The amount of the ingredients was arranged in such a
total volume in each tube was made up to 2 c.c. before the final incubation.

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[5] This emulsion of lipoid was anticomplementary in the dose 0.6 c.c.

TABLE 4.—Results of Experiments with Lipoids Extracted from the Tuberculin

		Amount of Serum Used for Titration															
		Case No.	0.1	0.05	0.02	0.01	0.007	0.005	0.003	0.002	0.0015	0.001	0.0007	0.0005	0.0003	0.0002	0.00015
A	0.0001 c.c. of pure lipoid and 0.03 c.c. of compl.	134	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	
		206	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH		
		219	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	
B	0.04 c.c. of Besredka's tuberculin and 0.03 c.c. of compl.	134	NoH	NoH	NoH	NoH	.....										
		206	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH	NoH		
		219	CH	.....													
C	0.04 c.c. of the lipoid from the tuberculin and 0.03 c.c. of compl.	134	CH	.....													
		206	CH	.....													
		219	CH	.....													
D	0.4 c.c. of the lipoid from the tuberculin and 0.03 c.c. of compl.	134	NoH	NoH	CH												
		206	SlH	CH													
		219	NoH	NoH	SlH	CH											

hemolysis should have taken place also with the smaller amounts of serum. In short, although the tuberculin contains lipoid, the amount of the latter contained in the dose of tuberculin used in the test as antigen, is too small to cause any lipotropic reaction with the syphilitic serums. Therefore, the syphilitic serums fixing complement in presence of tuberculin must also contain a tuberculous antibody as was proved in my former experiments.

Although the previous experiments proved quite convincingly the independent coexistence of the two antibodies in the same human serums, I thought the reproduction of this condition experimentally would supply a further interesting proof of the specificity of the fixation with the tuberculin. For this purpose I selected from a series of rabbits having experimental syphilitic orchitis those animals which gave a positive Wassermann reaction. These animals were also tested against Besredka's tuberculin and gave invariably negative results. A series of five normal rabbits was used as a control. As shown in Table 5, Rabbits 102, 105, 109, 111 and 117 gave positive Wassermann at the time of the experiment; Rabbit 118, although likewise infected, developed Wassermann reaction somewhat later. March 5, seven rabbits having experimental orchitis as well as three control rabbits were infected with tuberculosis, and March 15, all of them showed a positive reaction when tested against Besredka's tuberculin by the complement-deviation method. As can be seen from Table 5, three syphilitic rabbits, not infected with tuberculosis, as well as two normal controls, gave at the same time a negative tuberculin reaction. March 17, some of the rabbits, as shown in the table, received, intravenously salvarsan (0.04 gm. per kilogram) which injection was repeated March 21, and the animals were again tested by the complement-deviation method March 30. The animals that had received salvarsan lost their Wassermann reaction, but continued to give positive reaction with Besredka's antigen.[6]

An analogous experiment was performed also on a number of patients who came to the hospital for the treatment of syphilis, and who in addition presented positive serum reaction with Besredka's antigen. Of these cases, there were eight in whom tuberculosis was clinically suspected and in four out of these it was definitely diagnosed. In the remaining four, however, there was no clinical evidence of active tuberculosis though their von Pirquet test was positive. All these cases, as well as control cases presenting the Wassermann reaction only, were repeatedly examined during their treatment, and,

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[6] The weakening of the Wassermann reaction, March 30, in the Rabbits 100 and 117 (which did not receive any salvarsan), is due to spontaneous cure. They entirely lost their Wassermann reaction when tested April 10.

TABLE 5.—Showing Positive Reaction with Besredka's Antigen After Salvarsan Had Produced a Negative Wassermann

		Rabbits with Experimental Orchitis							Normal Rabbits (Controls)				
Rabbits No.		100	102	105	109	111	117	118	130	131	132	133	134
3/3	W. R. Tb. R.	+ —	+ —	+ —	+ —	+ —	+ —	— —	— —	— —	— —	— —	— —
3/5		Not	infect	ed	In	fected tuber	with culosis		Not in	fected	In tu	fected bercu	with losis
3/15	W. R. Tb. R.	+ —	+ —	+ —	+ <+	+ +	+ <+	«+ «+	— —	— —	— +	— <+	— «+
3/17-21		No salv.	Salvar intra	san ven.	Salva intra	rsan ven.	No sal	varsan	Salv. intraven.	No sal-varsan	No salv.	Salvar intra	san ven.
3/30	W. R. Tb. R.	<+ —	— —	— —	— +	— +	«+ <+	<+ <+	— —	— —	— +	— +	— «+

The sign + means positive reaction.
The sign <+ means weakly positive reaction.
The sign «+ means almost negative reaction.
The sign — means completely negative reaction.

whereas the Wassermann reaction disappeared after a certain period of time in at least fifteen cases, the tuberculosis reaction persisted.[7]

Having thus proved that, on the one hand, the positive reaction with tuberculin is not of a lipotropic nature, and that, when present in serums giving also positive Wassermann reaction, it may be shown to exist separately; and, on the other hand, having caused Wassermann reaction to disappear under treatment from the serums of the experimental animals and human beings giving both reactions without the disappearance of the tuberculosis reaction, I was convinced that complement fixation with Besredka's antigen indicates an independent tuberculous infection whether primary or secondary to other conditions.

These findings are to a great extent borne out also by clinical observations on over 600 cases comprising such widely varying pathologic conditions as typhoid, specific meningitis, tuberculosis, pernicious anemia, cancer, pneumonia, scarlet fever, diphtheria, syphilis, gonorrhea, trichinosis, and various surgical conditions, which were studied up to date. As the majority of cases included in this series was not selected especially but was taken at random as admitted to this hospital, the number of tuberculous patients among them was comparatively small. Through the kindness of Drs. Park, McNeil and Pease of New York I was enabled, however, to bring the total number of cases of known tuberculosis up to 174.[8] On account of the remarkably high percentage of positive tuberculosis reactions found among syphilitics from the beginning of this work,[9] I decided to subdivide all the cases, tuberculous as well as non-tuberculous, according to the positive or negative history of syphilis.

As can be seen from Table 6, among sixty-five cases of active tuberculosis the reaction was present in sixty-one; in the remaining four it was negative. Three out of these four cases were of tuberculous peritonitis (one of them terminating fatally) and in the other two the diagnosis was confirmed by Dr. Andrews from the examination of tissue removed at operation. The fourth case was that of a man in a far-advanced stage of lung tuberculosis with a very great number of tubercle bacilli in the sputum. Among the 27 cases which were clinically cured or on the way to recovery, 15 gave positive and 12 (44.5 per cent.) negative reaction. It is interesting to notice that among 108 cases of syphilis in which tuberculosis was or was not clinically suspected, there were 41 cases (38 per cent.) with positive reactions. Of the 18 cases showing positive reaction in which tuberculosis was originally not suspected, but syphilis was definitely diagnosed, in 3 cases tubercle bacilli have since been found in the sputum and in 2 others clinical proofs of tuberculosis were present on subsequent rigid examination by Dr. Boyce. It is needless to mention that, in the 15 cases of this series on whom the von Pirquet test was made, it was always positive. Out of 75 cases comprising different pathologic conditions except syphilis, in which tuberculosis was not suspected, 6 gave a positive reaction. In 2 cases, which came later to necropsy, Dr. V. L. Andrews found ample confirmation of the serologic diagnosis. Out of 220 cases comprising obstetrical, surgical and gynecological material in which neither tuberculosis nor syphilis was suspected, 18 gave positive reaction. Among these, 5 were in children under 5, with positive von Pirquet; in the remaining 13 cases it was impossible to find confirmation of the serologic diagnosis, because the patients left the hospital very soon after the blood examination was

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[7] I noticed in two cases some change in the strength of the tuberculous reaction under the influence of salvarsan treatment. Whether this change is due to the effect of salvarsan on tuberculous processes, or whether salvarsan may exert direct influence on the serum reaction, is a question studied at present in my laboratory. I wish to express here my indebtedness to Drs. Schildecker and Walker, who kindly offered their cases for the study of the effect of salvarsan treatment upon the deviation test with the tuberculin.

[8] This number comprised cases of active tuberculosis mostly. There were, however, among these twenty-seven cases in which, although there was a very definite history of tuberculosis, no active symptoms had been present for a number of years.

[9] Bronfenbrenner, J.: Proc. Soc. Exper. Biol. and Med., 1914, xi, 92.

TABLE 6.—Reactions in Tuberculous and Non-Tuberculous Syphilitic Cases

		Tb. R. +	Tb. R.—	Total	Percentage +
No syphilis suspected. Tuberculosis diagnosed or suspected.	Active tuberculosis at present	61	4	65	93.84
	Definite history of tuberculosis, but no symptoms at present (convalescents)..	15	12	27	55.5
	Tuberculosis strongly suspected, but not diagnosed definitely	36	14	50	72.0
	Tuberculosis diagnosed or suspected; also syphilis treated and untreated.....	23	9	32	37.96
	Syphilis treated and untreated. Tuberculosis not suspected	18	58	76	
No syphilis nor tuberculosis suspected.	Different pathologic conditions	6	69	75	8.0
	Controls: surgical, obstetric, gynecologic	18	202	220	
	Controls: cases without any history at the time of serum test; accidents.....	6	74	80	
	Total	183	442	625	

made. The same applies to the six positive reactions out of the 30 cases in which no history whatsoever was at hand at the time of the serum test. Thus from the material on hand it follows that, except for four cases in extremely advanced stages, the reaction was positive in all the cases of active tuberculosis; that the reaction was negative in about 90 per cent. of cases in which tuberculosis was not suspected, and that, as to the remaining 10 per cent. in which the serum findings were the first to call the attention to the possible presence of active tuberculosis, and in which subsequent clinical or pathologic examination was possible, a confirmation of the serum diagnosis was obtained in many cases though not in all. It is in these latter cases that I tried my serum skin-test, which in every case corroborated the previous serologic findings.[10]

Inasmuch as twelve apparently cured patients with tuberculosis, having given a negative serum-reaction, all continued giving a positive von Pirquet I thought it would be of interest to compare the two reactions.[11] The numbers in Table 7 represent the results of such a comparison on a series of 70 cases.[12]

As can be seen from this table, the von Pirquet test was negative in only nineteen cases and these consisted chiefly of children, twelve being under 5, five under 15 years and only two, adults. The remainder, mostly adults, gave a positive von Pirquet. The serum test, however, was positive only in 30 of the 70 cases. In 18 of the 21 cases in which the von Pirquet was positive and the complement-deviation test negative, there was no clinical evidence of tuberculosis whatsoeven; in the remaining 3 cases, however, the results of von Pirquet coincided with the clinical diagnosis of tuberculosis. One of these cases in which meningeal symptoms were present, proved later, however, not to be of a tuberculous nature, showing thus the correctness of the serologic findings against von Pirquet. The other two cases were cases of very advanced tuberculous peritonitis mentioned before. This comparison adds another very important proof of the value of the complement-deviation test in tuberculosis, especially in its active stages.

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[10] Though the number of cases to which this test has so far been applied by me is too small to claim for it unquestionable value, yet the results obtained are sufficiently constant to consider it as additional proof of tuberculosis with other evidences pointing in the same direction. The technic of this test is the following: Fresh serum from the suspected patient is mixed with tuberculin of Besredka in relation 10: 1, and 0.05 c.c. of this mixture is injected into the skin of a normal guinea-pig, after the mixture is allowed to stand at room temperature for three hours. Two or three hours after the injection — in case the serum used was tuberculous — the guinea-pig presents thermic reaction, and at the end of twenty-four (forty-eight) hours a local reaction similar to the tuberculin reaction follows. Each series of guinea-pigs is accompanied by a control guinea-pig receiving intradermally the double dose of tuberculin alone. In addition to this, as a control for the serum, each guinea-pig is injected on the side opposite to that of the test injection with the double amount of the suspected serum alone. The results are taken only in those cases where the controls do not show any reaction. This precaution is necessary because some serums show a tendency to cause a local irritation.

[11] Since last February I have been studying on a large series of infants, in collaboration with Dr. Pease, the comparative value of the von Pirquet and complement-deviation test. Though this study is incomplete as yet, it is already apparent that accepting the known diagnostic value of the von Pirquet test on infants, it offers a very favorable control to the serum test, thus adding an additional proof of the specificity of the complement-deviation test with Besredka's antigen.

[12] This group of individuals was not selected in any way, but simply represents the cases in which I was permitted to perform a von Pirquet test as a control to the serum test. The number of cases is comparatively small, but I was unable to have the von Pirquet performed on all cases on account of the desire to avoid the unnecessary inconvenience to the patient incidental to this test.

Inasmuch as, on the one hand, the complement-deviation test was negative not only in 92 per cent. of the cases in which tuberculosis was not suspected clinically, but even in 45 per cent. of convalescents, and, on the other hand, as the suspicion of this reaction being of a lipotropic nature had been excluded by the above-described experiments, the high percentage of positive reactions obtained among syphilitics in my series becomes very significant. The percentage of 37.9 of positive tuberculosis reaction among syphilitics (as shown in Table 6) should be, in fact, even higher, as in this table only those cases in which the history of syphilis was given on admission were classed in this group. Assuming, however, that the patients giving positive Wassermann reaction (see Table 8) were syphilitics even when their presence in the hospital was due to other causes, the number of syphilitics in this series of 625 cases is 108 (diagnosed as syphilis) plus 85 (giving positive Wassermann reaction whether or not syphilis was suspected at the admission), or, 193. Out of this number of syphilitics 83 gave positive tuberculin reaction (14 giving negative Wassermann and 69 positive Wassermann reaction) being about 43 per cent.; among these cases, however, tuberculosis was diagnosed or strongly suspected in 55 cases (46 with positive and 9 with negative Wassermann) leaving 28 (83 — 55) cases of syphilis in which tuberculosis was not suspected, or, as the total number of syphilitics in whom tuberculosis was not suspected is 138 (193 — 55), the rate of

TABLE 7.—Comparative Results of Serum and Von Pirquet Tests

Von Pirquet + Tb. R. +	Von Pirquet + Tb. R. —	Von Pirquet — Tb. R. +	Von Pirquet — Tb. R. —
30	21	0	19

positive tuberculosis reaction in syphilitics in whom tuberculosis was not previously suspected is about 20.3 per cent.

For non-syphilitics (taking as non-syphilitics all the persons in whom no history or symptoms of syphilis were found and the Wassermann reaction was negative), the corresponding numbers are the following: Total number is 625 — 108 (diagnosed as syphilitics) — 85 (syphilis not diagnosed, but Wassermann reaction positive) equals 432. Out of this number only 100 gave positive tuberculin reaction, or about 23.15 per cent. Among these, tuberculosis was diagnosed or strongly suspected in 80 cases, which leaves 20 (100 — 80) cases of positive tuberculin reaction out of 352 (432 — 80 diagnosed tuberculosis) cases comprising various pathologic conditions with the exception of syphilis, and practically normal controls, which makes less than 5.68 per cent.[13]

TABLE 8.—REACTIONS IN SYPHILITIC CASES NOT PREVIOUSLY SUSPECTED OF BEING TUBERCULOUS

	TB.+W.+	TB.+W.—	TB.—W.+	TB.—W.—	Total
Tuberculosis diagnosed or strongly suspected. (No syphilis suspected)	32	80	2	28	142
Tuberculosis diagnosed or suspected; also syphilis treated and untreated	14	9	4	5	32
Syphilis treated and untreated. Tuberculosis not suspected	13	5	15	43	76
Different pathologic conditions. No tuberculosis or syphilis suspected......	2	4	8	61	75
Controls (normal) obstetric, surgical accidents, no syphilis or tuberculosis suspected	6	12	22	180	220
Cases without any history or diagnosis at the time of the serum test	2	4	11	63	80
Total	69	114	62	380	625

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[13] This number probably is still smaller, as among those classed as non-syphilitics there may be a certain number of cases of treated or cured syphilis which on account of the absence of definite symptoms and in view of negative Wassermann do not confess a specific history. I had the opportunity of discovering by means of Noguchi's luetin test at least one such case in my series. After the patient under the weight of the evidence, admitted the infection 18 years before, it was duly transferred into the group of treated syphilitics in my series.

Thus the occurrence of tuberculosis, as estimated by the complement-deviation test, is very much higher among syphilitics than among non-syphilitics, as is shown in Table 9.

SUMMARY

The complement-deviation test with Besredka's antigen is not lipotropic in nature. Whenever present in serums possessing high lipotropic property, it depends on the presence of a separate antibody possessing its own index different from the lipotropic index in the same serum.

Serums possessing similar lipotropic indices may therefore have widely different indices when tested with Besredka's antigen.

TABLE 9.—Showing Greater Percentage Incidence of Tuberculosis Among Syphilitics Than Among Non-Syphilitics

	Tuberculosis Reaction Positive with Besredka's Tuberculin				
	Tuberculosis Clinically Diagnosed or Strongly Suspected	Tuberculosis Clinically not Diagnosed nor Suspected	Total Number of Positive Tb. R.	Average Percentage	
				Among All Cases	Among Non-Diagnosed as Tuberculous
193 syphilitics	55	28	83	43.0	20.3
432 non-syphilitics.	80	20	100	23.15	5.68
Total number of 625	135	48	183	29.28	9.79

When the serum deviates the complement in presence of both Besredka's tuberculin and pure lipoid antigen, each of the two antibodies can be exhausted from the serum independently of each other.

Besredka's tuberculin can be freed of its lipoid without losing its antigenic property.

The presence in the same serum of the property to fix complement with both antigens independently can be reproduced in experimental animals, and, when present in human beings (as well as in animals), the lipotropic antibody disappears under salvarsan treatment, whereas the tuberculous antibody persists.

The comparison of the results obtained by the complement-deviation test with those obtained by von Pirquet and their analysis in the light of the clinical histories of the cases points to the evident specificity of this complement-deviation test.

The findings of other workers who had the opportunity of using Besredka's antigen are even more convincing. Whereas, in my series, the percentage of positive reactions in cases of active tuberculosis was 93.84 per cent, Inman,[14] for instance, reports 95 positive reactions in 100 patients showing tubercle bacilli in the sputum. As to the cases, comparatively few in number, in whom, in spite of active tuberculous processes, the reaction gave negative results, the clinical picture of the cases as well as the data in my possession from the study of this reaction on experimental animals seem to suggest that in the latest stages of the disease the circulating antibodies seem to disappear from the blood.[15]

The question if the antibody is bound by the excess of the free circulating antigen (which seems most probable) is being studied at present in my laboratory by the method of Debré and Paraff.[16]

The high percentage of negative reactions among clinically nontuberculous (92 per cent.), together with the other proofs of the high degree of specificity of this reaction, seem to indicate that the comparatively frequent simultaneous occurrence of the complement-deviation test with Besredka's antigen and the Wassermann reaction in syphilitics is due to the fact that either syphilis as such or the antisyphilitic treatment markedly lower the resistance of the patients so as to make them either more susceptible to new infection of tuberculosis or to render them less resistant against the progress of this disease previously contracted.

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[14] Inman, Compt. Rend. Soc. de Biol., 1914, No. 4, p. 251.

[15] Bronfenbrenner, J.: Science, 1914, xxxix, 804; Proc. Soc. Exper. Biol. and Med., 1914, xi, 92.

[16] Debré and Paraff: Compt. Rend. Soc. de Biol., 1911, July 8, 16, 23, 28.

A CLINICAL STUDY OF TWO HEPATIC FUNCTIONAL TESTS

(GALACTOSE AND PHENOLTETRACHLORPHTHALEIN)*

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The object of this paper is to add further clinical observations to those already assembled on the subject of hepatic function. A voluminous literature has been assembled already on the subject both from an experimental and clinical standpoint. A very excellent general review of this is set forth in Holweg's[1] publication later reviewed and amplified by Riess and Jehn.[2] The more recent work has been presented by Rowntree, Hurwitz and Bloomfield.[3] In this study we have limited ourselves to the use of two tests, namely, the galactose test and the phenoltetrachlorphthalein test. These seemed to offer the greatest possibilities from a clinical point of view.

I. GALACTOSE TEST

The structural formula of d-galactose is $CH_2OH\ (CHOH)_4\ COH$. Its stereo-isomere is dextrose. It can be formed by hydrolysis from lactose. It rotates polarized light to the right. It ferments with greater difficulty than does glucose.

Relatively little experimental work has been carried out with galactose in connection with hepatic function. Sachs[4] showed that if the liver was removed from frogs a decreased tolerance to levulose was produced; however, no change in the tolerance to galactose was observed. Later Kausch and Socin showed that this was not true in dogs. Bauer's[5] later clinical observations confirmed these findings, thus showing that the ability to utilize galactose in men and dogs was different from that in frogs. Kausch and Socin[6] also showed that galactose with dextrose, saccharose, maltose and levulose must be considered as a glycogen-former. Hofmeister[7] showed, however, that the tolerance in dogs was a great deal lower for galactose than for the other sugars. Of interest is the work of Drauat,[8] who showed that the tolerance to galactose in a dog after an Eck fistula is reduced 79 per cent., while in the case of lactose it is reduced 42 per cent. More recently Fischler[9] has shown that after an Eck fistula in a dog the animal will tolerate the same amount of dextrose and levulose as before the operation; however, 80 per cent. of all ingested galactose appears in the urine. Drauat[8] concludes that the liver has a peculiar function in being able to utilize the galactose as no other organ. He states that a galactosuria would seem to indicate that there is a greater or less shunting off of the normal parenchyma of the liver or at least that the blood does not circulate through it. Roubitschek[10] has shown that, in rabbits with hepatic lesions produced by phosphorus, intolerance to galactose could be demonstrated. Wörner[11] made similar observations showing that the intolerance was in direct proportion to the degree of liver injury and independent of renal irritation. Roubitschek[10] also noted an increase in tolerance as more chronic lesions were produced. Of importance also is the work of Riess and Jehn,[2] in which they conclude from their experiments on dogs that the effect of mechanical obstruction of the common duct (ductus choledochus) per se causes no change in liver function as estimated by the galactose output. From an experimental standpoint, therefore, the use of galactose, as of dextrose and levulose, for a test of hepatic function is based on the power of the liver to change galactose as well as the other monosaccharids, to glycogen. The greater importance of the liver in the utilization of this sugar is a special argument for its use.

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* Submitted for publication June 17, 1914.

* From the Medical Service of the Peter Bent Brigham Hospital, Boston.

[1] Hohlweg: Deutsch. Arch. f. klin. Med., 1909, xcvii, 443.

[2] Reiss and Jehn: Deutsch. Arch. f. klin. Med., 1912, cviii, 187.

[3] Rowntree, Hurwitz and Bloomfield: Bull. Johns Hopkins Hosp., 1913, xxiv, 327.

[4] Sachs: Ztschr. f. klin. Med., 1899, xxx, 87.

[5] Kausch and Socin: Arch. f. Exper. u. Pharmak., 1893, xxxi, 398.

[6] Bauer: Wien. Med. Wchnschr., 1906, vi, 2537.

[7] Hofmeister: Arch. f. exper. Path. u. Pharmak., 1889, xxv, 240.

Bauer[5] in 1906 first utilized galactose as a clinical means for diagnosing liver diseases. The studies thus far carried out have not given uniform results. The findings of Hirose,[12] Frey[13] and others are of no value for comparative study, as these authors used different amounts of galactose. Of little interest also are the results of Strauss,[14] who made a comparative study of the uses of galactose and levulose. He used a smaller amount of galactose than employed by Bauer.[5] No definite conclusions can be drawn from his findings. Bauer gave his patients 40 gm. of galactose and considered an output above 3 gm. as pathological. He found that 90.6 per cent. of his thirty-two cases of cirrhosis of the liver, 100 per cent. of his forty-three cases of catarrhal

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8. Draudt: Arch. f. exper. Path. u. Pharmak., 1913, lxxii, 457.
9. Fischlet: Verhandl. d. Kong. f. inn. Med. in Wiesbaden, 1913.
10. Roubitschek: Deutsch. Arch. f. klin. Med., 1912, cviii, 225.
11. Wörner: Deutsch. Arch. f. klin. Med., 1913, cx, 293.
12. Hirose: Deutsch. med. Wchnschr., 1912, xxxviii, 1414.
13. Frey: Ztschr. f. klin. Med., 1911, lxxii, 383.
14. Strauss: Deutsch. med. Wchnschr., 1913, xxxix, 1780.

jaundice and 8.3 per cent. of sixty cases of localized conditions in the liver gave an abnormal output. Bondi and König,[15] using the same amount of galactose, found that 100 per cent. of eight cases of catarrhal jaundice put out more than 3 gm. Riess and Jehn[2] also gave 40 gm., but considered an output of 2 gm. as pathological. These authors found that 33.3 per cent. of fifteen cases of cirrhosis, 88 per cent. of seventeen cases of catarrhal jaundice, 25 per cent. of eight cases of cholelithiasis and 40 per cent. of five cases of icterus syphiliticus gave abnormal outputs. All of the five cases with tumors of the liver gave normal outputs. (Four of these were confirmed by autopsy.) Lattin[16] also, considering an output of 2 gm. as pathological, found that 10.5 per cent. of nineteen normals, 29 per cent. of seven cases of cirrhosis, 66 per cent. of three cases of catarrhal jaundice showed an abnormal amount in the urine. (It should be stated that one of these catarrhal jaundice patients was given the galactose late in the disease.) All of Lattin's eight patients with chronic passive congestion excreted less than 2 gm.

It is evident from the results of previous investigations that no far-reaching conclusions can be drawn. All authors seem to agree that a marked galactosuria in hepatic disease indicates the presence of a so-called catarrhal jaundice. Further clinical studies must be carried out before the value of the test under other conditions can be established.

The technic employed in this study has been similar to that advocated by Bauer.[17] Forty gm. of galactose are given by mouth at 6 a. m. in 200 or 300 c.c. of tea. The usual breakfast is given one hour later. The urine is collected for a period of six hours after the ingestion of the galactose at two-hour intervals. This urine is tested for sugar. The following tests have been employed: Fehling's, Nylander's and in some cases the fermentation test. The amount of sugar is estimated with the polariscope. The saccharimeter used for glucose determinations is employed. The specific rotation of galactose, according to Lippman, is 80.6 degrees, that of glucose 52.8 degrees. It is therefore necessary to divide the galactose readings by 1.4 as the rotation of galactose is this much greater than that of glucose. The chemically pure galactose (Merck) has been used. An output of above 2 gm. has been considered pathological.

In this study the cases have been classified according to the clinical picture as follows: (1) normal cases (Table 1); (2) cases of icterus catarrhalis; this group may include cases of cirrhosis of the liver with jaundice due possibly to a superimposed condition not unlike that of icterus catarrhalis (Table 2) ; (3) cases of cirrhosis of the liver without jaundice (Table 3) ; (4) cases with occlusion of the common or cystic ducts of long standing (Table 4) ; (5) miscellaneous hepatic conditions (Table 5). Twenty-nine cases have been studied.

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[15] Bondi and König: Wien. med. Wchnschr., 1910, ix, 2617.

[16] Lattin: Post-Graduate, 1913, xxviii, 368.

[17] Bauer: Deutsch. med. Wchnschr., 1908, xxxviii, 1505.

Little study has been made of the output of galactose in normal individuals. The results, based largely on negative findings and previous observations, show that in the majority of cases less than 1 gm. is excreted.

Four cases of icterus catarrhalis have been studied (Table 2). Seventy-five per cent. of these showed a very definite abnormal output. Case 10 was given the test during the late stages of the disease.

Eleven cases of cirrhosis of the liver have been studied (Table 3). Accurate determinations were impossible in one case, as the patient vomited after taking the galactose. Ten per cent. of the ten remaining showed an increased output. None of these patients were febrile or showed any evidence of jaundice. Fifty per cent. of them had very marked ascites, but showed results similar to those without ascites. Case 25 is of interest, as it represents clinically a very typical case of cirrhosis and hemachromatosis, but had a normal tolerance to galactose. Case 16 was controlled by autopsy and a very characteristic diffuse atrophic cirrhosis found. The diagnosis in Case 23 was confirmed at operation. With two exceptions this series of cases has been observed for several months and there is very little doubt clinically concerning the diagnosis.

Six cases with jaundice showing complete biliary stasis extending over a long period of time were given the test (Table 4). At the time of entrance some of these cases presented the diagnostic problem of the existence of a catarrhal jaundice. None of these cases showed an abnormal output. Necropsies were performed on Cases 29 and 32 and the diagnoses confirmed. In the case of No. 32, a cirrhosis of the liver and metastatic tumor nodules in the liver were found on histological examination. Notwithstanding this the galactose tolerance was normal.

In Group 5 (Table 5, miscellaneous cases) is the one case of exophthalmic goiter (Case 35). The output in this case was 2.5 gm.

Thirty determinations of galactose output have been made on the twenty-nine patients. The nausea and vomiting so common after the ingestion of the necessary amounts of other sugars as levulose are generally absent. We have had but a single case that had diarrhea after the ingestion of the galactose. The administration of a Dover's powder has been found entirely unnecessary. This has been advocated by some authors to combat a diarrhea that might be elicited and thus insure better absorption. This procedure adds an unnecessary complication. The technic of the test, together with the determinations of the sugar in the urine, is so simple as to require no further discussion. The estimation of the blood-sugar after the ingestion of the galactose has been suggested as a more accurate method to determine the hepatic function.

From the foregoing observations and from previous studies it seemed justifiable to assume that patients with icterus catarrhalis have an intolerance to galactose. On the basis of this fact we are led to consider this condition one primarily involving the bile capillaries within the parenchyma of the liver and not a condition due primarily to obstruction of the pars intestinalis of the common duct. In favor of this view is the fact that experimental ligation of the duct or occlusion of the duct by tumors does not cause a change in the sugar tolerance. Also this conception is further supported by the observations of Bauer, in which he showed gradual return to the normal tolerance as the condition improved. There is little evidence in support of the idea that cases of cirrhosis of the liver show a galactosuria after 40 gm. of galactose. The marked discrepancies in the results thus far published are possibly due to incorrect clinical diagnoses, lack of necropsy on the cases, or to different classification of cases. In reviewing the protocols of the cases previously reported it will be seen that many patients are said to have had marked jaundice. In many cases this may be due to a superimposed process not unlike that found in icterus catarrhalis. This later condition might well be considered to be a precursor of a cirrhosis. Some of the cases of cirrhosis of the liver with a low tolerance to galactose may also be associated with changes in the glands of internal secretion, as exophthalmic goiter, or with certain so-called neurasthenic conditions. These later conditions often show a galactosuria, as shown by the studies of Hirose.

II. PHENOLTETRACHLORPHTHALEIN TEST

The second test applied in this study is the phenoltetrachlorphthalein test. This compound was first prepared by Orndoff and Black.[18] Later it was studied by Abel and Rowntree.[19] These authors established its pharmacological properties with special reference to its action as a cholagogue. They noted that when given subcutaneously the drug escaped from the body only in the bile. It was on this property that its use as an hepatic test was based. Of interest pharmacologically and as a test are the very low toxicity of the dye and the fact that it is excreted in the bile in twenty minutes after the injection, and reabsorbed only to a slight extent by the large intestine.

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[18] Orndoff and Black: Am. Chem. Jour., 1909, xii, 349.

[19] Abel and Rowntree: Jour. Pharmac. and Exp. Therap., 1909, l, 233.

Whipple, Mason and Peightal,[20] and Whipple, Peightal and Clark[21] working on dogs, established experimentally the value of phenoltetrachlorphthalein as a means to determine hepatic function. These authors found in the normal feces the output is from 40 to 50 per cent. They produced hepatic lesions by the use of chloroform and phosphorus and found that following this a definite diminution of the output of the dye resulted. They state that "the drop in phthalein output appears to run parallel to the amount of parenchymatous injury." Of interest in this connection is the gradual increase in output as regenerative changes take place. In view of the work of Whipple aand Hurwitz[22] it is conceivable that after regenerative changes take place periods of hypersecretion follow.

In the light of the foregoing experimental findings, Rowntree, Hurwitz and Bloomfield[3] applied the phenoltetrachlorphthalein clinically as a test for hepatic function. These authors administered the dye as an aqueous solution of the disodium salt. Eighty determinations were made on sixty-seven patients. No very definite conclusions were drawn. These observers believe, however, that the normal output may vary from 30 to 52 per cent. Pyrexia apparently has no effect per se on the liver function. Secondary anemia must be extreme before the excretion of the dye is decreased. The liver function as indicated by this test becomes seriously affected only in those cases in which myocardial insufficiency is extreme.

We have followed essentially the technic as suggested by Rowntree.* The drug has been prepared fresh every two or three days. Four hundred mg., equal to 8 c.c. of the solution, have been given in 100 c.c. of normal saline. The normal saline solution has been used to avoid any hemolytic action such as had been recorded by Krumbhaar.[23] Before giving the dye, great care has been taken to insure excellent catharsis. Magnesium sulphate, from 15 to 20 gm. was given in the morning at 6, the injection at about 12; this was followed by 1 or 2 compound cathartic pills at intervals, depending on the result. Little attention thus far has been paid to the diet. It is my impression that patients on liquid diet do not make collections as satisfactorily as those on the regular "house" diet. The dye is given intravenously with a gravity apparatus. The stools are collected for the next forty-eight hours. As a matter of fact, we have found that there is practically never any further excretion after thirty-six hours. It is not unlikely

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20. Whipple. Mason and Peightal: Bull. Johns Hopkins Hosp., 1913, xxiv, 207.
21. Whipple, Peightal and Clark: Bull. Johns Hopkins Hosp., 1913, xxiv, 343.
22. Whipple and Hurwitz: Jour. Exper. Med., 1911, xiii, 136.
23. Krumbhaar: Jour. Am. Med. Assn., 1914. lxii, 992.

* We are indebted to Dr. L. G. Rowntree for the phenoltetrachlorphthalein used in these studies.

that the test could be carried out satisfactorily in a period of twenty-four hours. The personnel of the ward is carefully instructed concerning the collection. On some occasions transfer of excreta from one container to another has caused low outputs due to the loss of feces left in the first container. The precautions taken for careful collections cannot be overemphasized. With this in view the test has been carefully demonstrated to pupil nurses. The stools are tested separately for the presence of phenoltetrachlorphthalein by adding a drop of alkali to a few drops of the diluted feces. If the specimen turns to a pink or reddish color it is saved. The total excreta amount on an average to from 1,000 to 1,500 c.c. Exact dilution to either amount is made; this is shaken thoroughly for about five minutes, depending somewhat on the consistency of the specimens. If a shaker is not at hand the shaking may be satisfactorily done by placing the excreta in a 2-liter bottle and shaking it manually. One-tenth of this amount is now taken, and to this is added about 5 c.c. of a 40 per cent. solution of sodium hydroxid. This amount is by no means fixed. Sufficient alkali is added to insure a maximum intensity of color. As the reaction of specimens varies, the amount of alkali varies. The maximum color can be easily determined by taking small portions in test-tubes and adding alkali until the maximum color appears. An excess of alkali causes a pinkish shade. The aforesaid amount of feces is now diluted to 1,000 c.c. It is shaken for two or three minutes. One hundred c.c. of this are now taken and a saturated solution of basic lead acetate added, 5 c.c. being usually the correct amount. We have found that it is safe to add until no further precipitation of the bile pigments occurs. As pointed out by Whipple, the lead acetate often lowers the reading 2 or 3 per cent. A few cubic centimeters, usually about 5, of 40 per cent. sodium hydroxid are now added until the maximum color is established. Here again great care must be taken not to add an excess of alkali. Small amounts are taken in test-tubes and tested out at various degrees of alkalinity until the maximum color is obtained. The solution is diluted to 200 c.c., filtered and read in the Rowntree-Geraghty modification of the Autenrieth-Könisberger colorimeter. The percentage is read by making up a standard solution as follows: 0.4 c.c. of the original solution of tetrachlorphthalein are diluted to 1,000 c.c. after the addition of a few drops of 40 per cent. sodium hydroxid. We have always used a standard made from the same solution as used for injection. We have experienced little difficulty in obtaining a color that can be read accurately. We have found that the readings are more satisfactory if artificial illumination is used.

After injection of the dye we have observed but one definite reaction. This was in Case 27. The patient was convalescing from an operation and the exact cause of the fever (101 C.) after the injection is not definite. In five cases we have noted local thrombi at the site of injection. These have had no relation to the disease of the patient and have caused very little discomfort. The same condition is often observed after the injection of salvarsan.

In the selection of cases we have endeavored as far as possible to employ such as present the question of some hepatic disease as a definite or very possible diagnosis. A division of cases similar to that in the galactose studies has been made. Where possible, the same patient has been given both tests. The dye has been given fifty times to 39 patients. Owing to faulty collections, the results of tests made on only thirty-three cases have been reported.

On studying Table 1 it will be seen that seven patients have been selected who unequivocally were not suffering clinically from any hepatic disease. These showed an output ranging from 30 to 44 per cent. A number of other patients, later shown not to be suffering from any hepatic disease, gave similar results. Case 5 later came to necropsy and showed normal hepatic findings histologically. This patient had an output of 32 per cent.

The cases of icterus catarrhalis (Table 2) and those showing complete biliary stasis of long standing (Table 4) are not adapted for study of hepatic function by means of this test. (The recognition of the biliary obstruction should be made by the determination of bile in the urine and in the blood.) It is self-evident that incomplete as well as complete biliary obstruction invalidates the results of the test.

Case 9 (Table 2) is of some interest. It shows the steady increase in output of the dye from a period of total biliary obstruction. At a period, however, when there was no evidence of the catarrhal jaundice clinically, when there was no evidence of bile in the blood or urine, the output continued low. A temporary injury to the liver cells undoubtedly persisted after the obstruction had disappeared. This is quite in harmony with our interpretation of the disease from studies with the galactose test.

Eleven cases of cirrhosis of the liver (Group 3) have been studied. None of these cases showed any evidence of biliary stasis. Five, or 49 per cent. of the total number of cases, showed a distinctly low output. One case (No. 16) in which the collections were excellent and the output was 19 per cent., was carefully controlled by autopsy findings. A typical atrophic "hobnail" liver was found. Histologically, this showed a very diffuse atrophic type of cirrhosis, there being great irregularity in the size of the lobules and no marked evidence of regenerative changes. The remaining five cases in this group showed a normal to relative high output.

Of interest from a prognostic standpoint is Case 28 among the group with biliary stasis. This case was considered to have a tumor involving the head of the pancreas with a question of metastasis in the liver. A cholocystduodenostomy was done. A few days later the phthalein output was practically normal, showing that there were not extensive lesions in the liver. This was actually the case clinically. The patient was seen thee months later; he had gained 10 kg. in weight, and his jaundice had totally disappeared. In this case the test also gave an easy method to determine the presence of bile in stools that contain blood. A few cubic centimeters of the dye are injected intravenously and the feces collected. From these the bile and blood pigments are removed by precipitation with lead acetate and filtration, and the filtrate is made alkaline with sodium hydroxid. The presence of the color due to the dye indicates that bile has entered the intestine.

The cases in Group 5 (miscellaneous) show such varied conditions clinically that a discussion of the results is hardly warranted. Cases 33 and 40 show results similar to Rowntree's two cases of neoplasm involving the liver. Unfortunately, the diagnosis in our cases was not controlled by operation.

The results of the studies with phenoltetrachlorphthalein show, as Rowntree pointed out, that the normal output of the dye in the feces is about 35 per cent. The inapplicability of the test to cases with evidence of biliary stasis makes its scope limited. In the light of the results obtained in cases of cirrhosis it must be borne in mind that many cases are associated with periods of regeneration of liver cells and at times might well be expected to show a normal or increased function. The experimental work of Whipple is in harmony with this conception.

CONCLUSIONS

1. The galactose test may be of great value in differentiating icterus catarrhalis from other hepatic conditions showing biliary stasis.

2. The galactose test is rarely positive in cases of cirrhosis of the liver showing no evidence of biliary stasis.

3. There is no apparent relation between the excretion of galactose and phenoltetrachlorphthalein.

Further studies must be undertaken before any definite conclusions can be drawn concerning the use of phenoltetrachlorphthalein as a hepatic functional test.

Judging from the experimental studies and from the clinical observations noted up to this time, it would seem that a more exhaustive application of the test is justified. It offers a means of diagnosing certain cases of cirrhosis of the liver and of neoplasms of this organ. The results obtained may also be of some prognostic value.

PROTOCOLS

Case 1.—Diagnosis: Chronic cardiac disease; mitral stenosis.

This patient's history essentially negative except for an attack of rheumatic fever at 9. His present complaints, shortness of breath and precordial pain.

His heart was found to be enlarged; there were signs of mitral stenosis and auricular fibrillation. The abdominal and liver findings were not remarkable; there was no edema. Except for a tonsillectomy his condition was uneventful. He was discharged after forty-three days, improved.

Clinical Pathology: Findings essentially negative.

Phenoltetrachlorphthalein, 44 per cent.

Note: Test given when patient was up about ward with no subjective symptoms.

Case 2.—Diagnosis: Arteriosclerosis; myocarditis; angina pectoris.

This patient had no alcoholic history and had always been in excellent health. He came to the hospital because of attacks of precordial pain, pain in shoulders and dyspnea.

His heart enlarged to the left; there were no murmurs; except for general arteriosclerosis his physical examination not important. His liver not enlarged. The abdominal findings not remarkable.

Clinical Pathology: Blood: findings normal. Wassermann blood reaction negative. Stool: findings normal. Urine: findings normal.

Phenolsulphonephthalein, 60 per cent. in two hours. Phenoltetrachlorphthalein, 40 per cent.

Note: After the dye was given patient vomited repeatedly. In my opinion there were definite gastric symptoms before the injection and these were accentuated by the catharsis.

Case 3.—Diagnosis: (Syphilis of aorta) aortitis syphilitica; chronic myocarditis.

This patient had no alcoholic history. Previous to his present illness he had fourteen attacks of cardiac decompensation. there was marked dyspnea, cough and orthopnea. He came to the hospital with these symptoms.

His physical examination at the time the test was performed showed no signs of cardiac decompensation, his heart was enlarged, he had a dilated aortic arch. His liver was not enlarged; he had no edema. He was up about the ward.

Clinical Pathology: Findings essentially negative except for a positive Wassermann (blood). The renal tests showed normal findings.

Phenoltetrachlorphthalein, 41 per cent.

Case 4.—Diagnosis: Carcinoma of breast.

Nine years ago this patient had a radical operation for a scirrhous carcinoma of her left breast. Eight years later she noted a lump in this region. She had no subjective symptoms.

Except for the small nodules over the site of the previous operation the physical findings negative.

Clinical Pathology: Findings normal.

Phenoltetrachlorphthalein, 32 per cent. (in feces).

Case 5.—Diagnosis: Aortitis; aneurysm of thoracic aorta.

Patient's family history unimportant. He had exanthems in childhood; also pneumonia one year ago. He had nocturia, subject to dizzy spells, drank about two "whiskies" a day. He came to the hospital because of a gnawing pain in lower abdomen of ten weeks' duration. He vomited a few times. The pain had been fairly constant. Patient lost about 25 pounds during the past year.

Physical examination showed a rather poorly nourished, pale man. There was slight tenderness in epigastric region; an indefinite mass felt. The liver not enlarged. There was no evidence of fluid in the abdomen. Patient died suddenly; ruptured aneurysm was found at necropsy. The microscopic hepatic findings were negative.

Clinical Pathology: Findings unimportant except for positive Wassermann.

Phenoltetrachlorphthalein, 32 per cent. Galactose, no grams.

Case 6.—Diagnosis: Fibromas with pigmentation (von Recklinghausen's disease).

This patient had always enjoyed excellent health. He made very poor progress in school. He came to the hospital because of symptoms referable to hyperchlorhydria.

He showed numerous congenital defects beside the presence of numerous nodules over the body.

Clinical Pathology: Findings normal.

Phenoltetrachlorphthalein, 34 per cent.

Case 7.—Diagnosis: Pleurisy, chronic.

This patient used alcohol in great excess. Except for an attack of pleurisy eight years ago his history unimportant. He came to the hospital complaining of a cough and pain in the right side.

Physical examination showed signs of a fibrinous pleurisy over the right lower lobe. His course was afebrile; entirely uneventful.

Clinical Pathology: Urine showed a small trace of albumin and many white blood-cells. Phenolsulphonephthalein test, 59 per cent. output in two hours. Stool and blood showed essentially negative findings. Phenoltetrachlorphthalein, 36 per cent.

Case 8.—Diagnosis: Disseminated sclerosis.

This patient's personal and past histories unimportant. His gait became progressively more unsteady for the past three years, the ataxia was so marked that he became practically helpless.

The case was typical of multiple sclerosis, with intention tremor, nystagmus, scanning speech, exaggerated knee-jerks, ankle-clonus and Babinski. There was no sensory involvement.

Clinical Pathology: Findings normal.

Galactose excreted, 1.19 gm.

Case 9.—Diagnosis: Catarrhal jaundice.

Complaint: "Pain in stomach and yellowness." Six weeks ago he had intermittent dull, well localized epigastric pain, accentuated by stooping forward. His sleep had not been interrupted. His pain had no relation to meals. About five weeks ago he began to vomit, generally about half an hour after meals. This continued up to one week ago. Vomitus contained no blood. Patient did not think it contained bile. His appetite remained good, his bowels regular. One week ago his eyes and skin became yellow, his stools clay-colored. His urine had been dark-colored for three or four weeks.

He was a well-nourished and developed young man. His skin light yellow in color, also the sclera. The abdomen showed no areas of tenderness, no muscle spasm. The liver dulness extended from the fifth rib to 1 cm. below the costal margin in the nipple line. The edge felt 3 cm. below the costal margin in the right nipple line. Patient's course in the hospital was entirely uneventful. He had practically no subjective symptoms. He was observed about four weeks and discharged well.

Clinical Pathology: Urine contained bile until shortly before discharge. Urobilinogen was increased. Stools on entrance were clay-colored (contained bile at time of last test). Galactose, 2.6 gm., given at admission.

Phenoltetrachlorphthalein: Feces, 0; urine, 6 per cent., two days after admission; feces, 10 per cent.; urine, 0, ten days after admission; feces, 20 per cent.; urine, 0, three weeks after admission.

Case 10.—Diagnosis: Cirrhosis of liver; catarrhal jaundice.

This patient had been an habitual drinker. He had always been a remarkably healthy individual. He came to the hospital because of swelling of his legs. There had been some epigastric discomfort of late; otherwise no subjective symptoms.

His skin and sclera yellow-tinged; the liver markedly enlarged. There was possibly free fluid in the abdomen. There was edema and also a purpuric discoloration of the legs. After about two months the jaundice practically disappeared. In six months, however, patient came to the hospital again. This time he was much more jaundiced. His liver was still very much enlarged. His condition on both admissions was febrile for a number of weeks.

Clinical Pathology: Renal tests normal. Urine contained a slight trace of albumin and much bile during his last admission. Urobilinogen much increased. Blood: normal findings. Wassermann blood reaction negative. Stool often light-colored and evidently contained reduced amount of bile.

Galactose, first admission. 0.18 gm.; second admission, 1.7 gm.

Phenoltetrachlorphthalein test (second admission), trace in feces.

Case 11.—Diagnosis: Jaundice, catarrhal; cirrhosis of liver (?)

There was a *definite alcoholic history*. Patient used malt liquors, gin and wine, apparently often in excess. Her general health had always been good. She had never been seriously ill, been jaundiced or had any previous gastric symptoms. Five weeks before admission patient had a smothering sensation about the heart; about two weeks later had an attack of nausea and vomiting. Simultaneously the sclerae became yellow. The vomiting persisted up to admission, she had no abdominal pain.

On physical examination the skin was lemon yellow in color; the sclera also yellow. There was apparently moderate enlargement of the liver; otherwise the findings negative. Patient was observed about three weeks, her jaundice and vomiting cleared up in that time.

Clinical Pathology: Urine: bile present. Urobilinogen increased. Feces: bile present. Wassermann positive. Blood: findings negative.

Galactose, 4.3 gm.

Case 12.—Diagnosis: Catarrhal jaundice.

The patient's past and personal histories unimportant. About six weeks before admission she began to vomit; this persisted up to the time of admission. She had been nauseated and complained of epigastric pain almost constantly. She had not been jaundiced.

Her physical examination showed a slightly enlarged heart. The abdominal findings normal. The liver not enlarged. Her stay in the hospital was uneventful.

Clinical Pathology: Urine amber-colored, a slight trace of albumin; bile present. Stools contained no bile and were clay-colored on certain examinations.

Phenoltetrachlorphthalein output, 10 per cent. (feces clay-colored).

Note: Patient's stay in the hospital was so short that repeated tests could not be made.

Case 13.—Diagnosis: Cirrhosis of liver (?); catarrhal jaundice.

This patient had always been in excellent health. He had had no previous illness; there was no alcoholic history. For the past three months patient said he had had indigestion, complained of eructation of gas, of a gnawing sensation in the epigastrium, some nausea and persistent vomiting for the past thirty-six hours. His skin had been yellow for the past two weeks.

On physical examination one noted icterus of the skin and sclerae. Save for marked enlargement of the liver, the abdominal findings were negative. The edge of the liver sharp, the surface smooth. Patient's course in the hospital was entirely uneventful, his jaundice disappeared, he had no subjective symptoms after one week.

Clinical Pathology: Urine: bile present, large amounts of urobilinogen. Blood shows normal findings.

Galactose, 3.9 gm.

Case 14.—Diagnosis: Cirrhosis of liver; chronic nephritis (?).

This patient had an alcoholic history. About two years ago she first became dyspneic on exertion. Associated with this were edema of the feet, nycturia and polyuria. Her abdomen had been distended for the past year. She had had constant nausea for the past two weeks, some headache.

Her facies markedly alcoholic, her heart moderately enlarged; her blood-pressure 180 systolic, diastolic 95. She was obese. Her abdomen showed no signs of free fluid; the upper border of the liver began at the level of the fifth rib and extended 10.5 cm. below the costal margin in the right nipple line. The edge smooth, rounded. Patient's condition improved subjectively while in the hospital.

Clinical Pathology: Renal test, phthalein 60 per cent. in two hours. Urinary findings normal.

Galactose, 0 gm. Phenoltetrachlorphthalein, first test (feces), 22 per cent.; second test (feces), 21 per cent.

Case 15.—Diagnosis: Nephritis, chronic; cirrhosis of liver.

Patient had no previous illness of note. He always used alcohol, two or three glasses of whisky and two or three glasses of beer a day. For the past year he complained of swellings of his legs. He noticed swelling of his abdomen for the past six months; associated with this there had been moderate dyspnea. Patient markedly emaciated. There was marked edema of the ankles, legs, scrotum and sacral region. There were signs of fluid in the abdominal and pleural cavities (verified by repeated paracenteses). There was some cardiac enlargement; a slight hypertension. He was observed about four months. Tappings, limitation of fluids, Karell diet, diuretin, theocin, digitalis and sweat baths gave no relief.

Clinical Pathology: Phenolsulphonephthalein from 40 to 52 per cent. in two hours (repeated tests). No nitrogen retention. Slight sodium chlorid retention. Normal outputs of sodium iodid and lactose. A normal specific gravity of urine, always a trace of albumin with many hyaline and granular casts. Ascitic fluid: characteristics of a transudate.

Liver tests: Galactose after 40 gm.; 0.4 gm. excreted. First phenoltetrachlorphthalein, 22 per cent. recovered; second, 20 per cent. recovered.

Case 16.—Diagnosis: Cirrhosis of the liver.

Patient came in with complaint of "tightness" in the abdomen, especially on the right side. This was of four months' duration. He was constipated; he had no other subjective symptoms; however, he had not worked since the onset of his illness.

Physical examination showed a moderately well-nourished and developed Chinaman. There was no evidence of jaundice. There were signs of fluid in the pleural cavity, marked ascites. Of note was the enlargement of the superficial veins extending from the axilla down. The liver edge could not be felt, before or after tapping.

Abdominal paracentesis was performed on twelve different occasions. About 5 or 6 liters of fluid recovered each time; had the characteristics of a transudate. Inoculation of guinea-pigs with the fluid gave negative results.

Microscopic examination of liver taken from operation showed typical lesion of a diffuse atrophic cirrhosis.

Clinical Pathology: Wassermann reaction of blood, urinary findings, kidney function tests, stool examination and blood examination gave negative findings except for a high (108) urobilinogen content in the urine.

Phenoltetrachlorphthalein, 7 per cent. (poor collections.) Second phenoltetrachlorphthalein, 19 per cent. (excellent collections). Galactose 0.9 gm.

Case 17.—Diagnosis: Cirrhosis of liver.

Since the age of 20 patient drank three or four glasses of beer and one or two of whisky a day. More recently he drank ten or twelve whiskies a day with additional beer. He had measles and two attacks of pneumonia, but his general health had been good. He came into the hospital complaining of swelling of the legs, dizziness, dyspnea on exertion, a dull pain in the lower abdomen. He vomited very often in the morning; marked anorexia.

His face was deep red; there was dilatation of the superficial veins of the face. There was no jaundice. His abdomen was prominent; on admission there were signs of fluid in the abdomen; this was not verified later by paracentesis. His liver extended from the fifth intercostal space to 10 cm. below the costal margin. Palpation of the edge disclosed nothing remarkable. The superficial veins over the left abdominal half are prominent; there is edema of the ankles.

The patient remained in the hospital nineteen days; was discharged greatly improved, with no evidence of abdominal fluid, no edema.

Clinical Pathology: Wassermann negative. Urine: high specific gravity, no bile, albumin, small trace; occasional granular cast. Urobilinogen ten times normal amount. Feces and blood normal.

Liver: Phthalein, 40 per cent. recovered in feces. Galactose, 0.4 gm.

Case 18.—Diagnosis: Nephritis, chronic. Cirrhosis of liver (?).

This patient used liquor in great excess. He had typhoid fever; his general health good. He came to the hospital complaining of headache and vomiting. He had no dyspnea. Moderate nycturia.

Physical examination essentially negative. He had a hypertension — 190 systolic, and slight enlargement of the liver.

Patient's stay in the hospital was uneventful.

Clinical Pathology: Renal test, phenolsulphonephthalein from 30 to 42 per cent. in two hours (repeated). Urine showed a trace of albumin, many hyaline casts, rare red blood-cells, no bile. Blood: findings normal.

Phenoltetrachlorphthalein, first test, feces 19 per cent.; second test, 25 per cent.

Case 19.—Diagnosis: Nephritis, chronic; cirrhosis of liver (?); frontal sinusitis.

Patient's history is markedly alcoholic, 1 quart of whisky a day; three "whiskies" before breakfast. "Dry heaves" every morning. He had been working in a brewery for the past seven years, had no previous illness except measles. He complained of headaches, swelling of the ankles, face and hands, vomiting, had three hemoptyses. There had been moderate dyspnea.

Physical examination showed an enlarged heart, a slight hypertension. Liver enlarged; the upper border at the level of the upper border of the sixth rib in the right nipple line, the edge 4.5 cm. below the costal margin, no edema, no icterus.

After about three weeks' stay in the hospital his subjective symptoms disappeared; his physical examination remained unchanged.

Clinical Pathology: Urine showed a trace of albumin and many hyaline and granular casts; no bile. Phenolsulphonephthalein test showed 45 per cent. excretion in two hours. Blood: findings normal.

Liver tests: Galactose, 0.7 gm. Phenoltetrachlorphthalein, 20 per cent. (collections excellent).

Case 20.—Diagnosis: Cirrhosis of the liver; catarrhal jaundice.

There was a marked alcoholic history, seven or eight glasses of whisky a day. Patient had no definite history of any previous illness. About three weeks before entrance his eyes became yellow. He spat blood on a few occasions; had marked malaise, no other subjective symptoms.

His skin and sclera were icteroid in color. His liver began 3 cm. below right nipple and extended 5 cm. below costal margin in right nipple line. The edge hard and regular. No evidence of fluid in the abdomen. His stay in the hospital was uneventful. His icterus entirely disappeared.

Clinical Pathology: Wassermann negative. Urine: no bile. Blood normal. Stool normal.

Galactose, 0.94 gm.

Case 21.—Diagnosis: Cirrhosis of the liver.

Patient always been in excellent general health. He had been a moderate user of alcohol, one or two glasses of whisky, three or four glasses of beer a day. He had measles, scarlet fever and small-pox as a child. He had "indigestion" for the past five years. He had heartburn, eructations, etc., without gastric pain, vomiting or hematemesis. There had been moderate edema of the ankles for five years, also constipation with hemorrhoids. There had been no jaundice. For the past five weeks patient's abdomen increased in size, until there were such marked distention that he experienced considerable shortness of breath.

On physical examination a fairly well-developed and nourished man. There was no abnormal pigmentation of the skin. He had moderate cardiac enlargement, a systolic blood-pressure of 220. His abdomen greatly distended. There were evidences of fluid. The liver not enlarged; the edge easily felt after paracentesis, firm, nodular and rounded. In the region of the epigastrium a firm, rounded, small, hen's egg-sized mass, that moves with inspiration.

Clinical Pathology: Roentgen examination of the stomach gave negative findings. Patient showed a secondary anemia, a negative Wassermann reaction, normal urinary findings, negative findings in stools. The phenolsulphonephthalein output in the urine varied from 21 to 51 per cent. The ascitic fluid was greenish yellow; it contained 0.6 per cent. albumin and 500 cells per cubic millimeter.

Phenoltetrachlorphthalein, 36 per cent.

Case 22.—Diagnosis: Cirrhosis of liver.

This patient had a marked alcoholic history. He drank from six to ten glasses of whisky daily, also additional beer. Except for a history of typhoid fever seven years ago he enjoyed excellent health up to the present illness. For the past four weeks he had recurrent hemoptyses, morning nausea and sour eructations.

On physical examination his heart was found to be very much enlarged (17 cm. to left of midsternal line in the sixth interspace). There was a murmur of mitral insufficiency. The abdomen is protuberant; the superficial veins of the abdomen dilated. There was dulness in the flanks, but no fluid wave demonstrable. The liver dulness extended from the fifth interspace to 8 cm. below the costal margin in the right parasternal line. The edge felt rounded, but smooth. There was no edema of the extremities.

Clinical Pathology: Urine: normal findings; other findings normal.

Phenoltetrachlorphthalein, 46 per cent. Galactose, 2.21 gm.

Case 23.—Diagnosis: Cirrhosis of the liver.

This patient denied the use of alcohol in any form. In September, 1912, patient first noted enlargement of her abdomen. This persisted, and she was tapped thirteen times. She complained of severe pain in the abdomen.

Of importance, on physical examination, were the abdominal findings. There was marked distention, shifting dulness; a fluid wave was made out. The patient was not jaundiced.

Patient was transferred to the surgical service where an Eck fistula was attempted. This was not carried out on account of the presence of hepatic adhesions. The liver was found to be small and retracted upward. Its surface was irregular; had the characteristic "hobnail" appearance. An omentopexy (modified Talma operation) was performed. Patient's condition was unrelieved. She died about two and one-half months after the operation. No necropsy was secured.

Clinical Pathology: Ascitic fluid, specific gravity, 1.012, clear, contained few mononuclear cells, 1.5 per cent. albumin. Urine and blood: findings essentially negative.

Galactose Test: Forty gm., given on two different occasions; gave a slight trace in the urine each time. The test was not satisfactory, however, as on one occasion patient vomited soon after taking the galactose, on the other occasion there was considerable diarrhea.

Phenoltetrachlorphthalein Test: This was the first test performed and some difficulty was experienced in reading the results. It is safe to say that a relatively high percentage was recovered — 50 per cent.; no dye was found in the urine.

CASE 24.—Diagnosis: Syphilis of the liver (?); cirrhosis of the liver.

This patient's past and personal histories unimportant. She had pain for many years in the upper right abdominal quadrant. About eight months ago her abdomen began to swell and she was tapped repeatedly during the following months. There had been no jaundice, no edema. She lost much weight.

On physical examination the hepatic dulness began at the fourth rib and was continuous with the abdominal dulness. After paracentesis the liver edge was felt 8 cm. below the costal margin in the right nipple line. The patient's condition became progressively worse. Antisyphilitic treatment had been carried out.

Clinical Pathology: Urine: findings normal. Blood: Wassermann positive. Phenoltetrachlorphthalein, 45 per cent.; galactose, 1.4 gm.

CASE 25.—Diagnosis: Hemachromatosis; cirrhosis of the liver.

Patient 68 years of age. Twenty-three years ago he fell injuring his abdomen (the exact nature of the injury not ascertained). Since this time he thought his skin had been very dark. He drank two or three "whiskies" a day for many years. He came into the hospital because of sensation of nausea and general malaise. Also his legs had been swollen.

On physical examination his skin everywhere dark brown in color. It had a definite bronzed appearance. There were purpuric spots over the extremities. His abdomen not distended. His liver edge felt 6 cm. below the costal margin in the midclavicular line. There was a large elevated area over the mass that corresponds to the liver. The liver apparently enlarged. Patient showed evidence of much loss in weight.

Clinical Pathology: Urine: Findings normal. Other findings not important. Section from skin showed presence of pigment (not hemosiderin).

Galactose, 0.6 gm.; phenoltetrachlorphthalein, trace in feces.

CASE 26.—Diagnosis: Cirrhosis of liver.

This patient forty years of age, used liquor in great excess for many years. He always enjoyed excellent health. About three months ago he suddenly became jaundiced; this disappeared in two weeks. Associated with this he had anorexia and pain in epigastrium. Six weeks ago he developed ascites and edema of legs. He had been dyspneic and had attacks of epistaxis.

Of importance on physical examination were the signs of fluid in the pleural and abdominal cavities and edema of legs. He was not jaundiced. There were distended superficial abdominal veins.

Clinical Pathology: Urine: normal findings, no bile. Blood: normal findings. Ascitic fluid, characteristics of a transudate.

Galactose, trace. Phenoltetrachlorphthalein, feces, 36 per cent.; urine, trace.

CASE 27.—Diagnosis: Cholelithiasis (common duct?); carcinoma of head of pancreas.

At times patient used alcohol to excess. He complained of dull epigastric pain, anorexia, frequent attacks of feeling cold and feverish, also of loss of weight and itching of the skin.

The skin and sclera had a marked icteric tint. The liver markedly enlarged. At times a mass corresponding to the gall-bladder had been palpated.

This patient was observed for one year. At times the icterus cleared markedly but recurred. His general condition remained fairly good. He refused operation.

Clinical Pathology: Renal tests normal. Urine always showed a slight amount of albumin and often trace of bile and many granular casts. Stools, in general clay-colored, at times, however, normal. Blood: evidences of a mild secondary anemia.

No phthalein or galactose recovered.

Case 28.—Diagnosis: Carcinoma of head of pancreas (?).

For the past seventeen years patient used no alcohol. His past history unimportant. About ten weeks before admission he complained of a burning sensation in the epigastrium; three weeks after this time he became yellow; the jaundice persisted. There had been no abdominal pain. On one occasion he was feverish. There had been marked loss of weight and much pruritus.

On physical examination he showed evidence of much loss of weight; his skin is jaundiced, lemon yellow. The sclera were intensely jaundiced. The liver markedly enlarged; a mass corresponding to the gall-bladder palpated. There was no abdominal tenderness. X-ray examination, also proctoscopic examination, showed negative findings.

He was operated on December 30. The condition at operation was thought to be carcinoma of the head of the pancreas. A cholecystenterostomy was performed. Patient made a satisfactory postoperative recovery. Bile again appeared in the stools; the jaundice disappeared to a great extent. The recovery was so remarkable that the original diagnosis seemed questionable.

Clinical Pathology: Urine shows presence of bile. Urobilinogen present in traces. Stool clay-colored; a negative guaiac. Wassermann blood reaction negative.

Galactose, 0 gm. Phthalein none before operation; 29 per cent. after operation.

Case 29.—Diagnosis: Carcinoma of head of pancreas.

This patient, 52 years of age, had vomited persistently for three months, complained of pain in his abdomen, lost weight and been jaundiced for a number of weeks.

Physical examination showed very marked jaundice of the skin and sclerae. A mass felt which extends 2 cm. below the umbilicus; hard, rounded and undoubtedly corresponded to a lobe of the liver. A mass corresponding to a distended gall-bladder was made out.

An exploratory laparotomy was performed. Patient died four days after the operation from hemorrhage. At autopsy a carcinoma of the head of pancreas was found. This had caused compression of the bile-ducts.

Galactose 0 gm.

Case 30.—Diagnosis: Cholelithiasis.

This patient had pertussis, diphtheria and measles in childhood. At the age of 12 she was jaundiced for several weeks. She had malaria twenty-three years ago. She had attacks of severe diarrhea, also attacks of "gastric distress," especially during the past year. On one occasion she had sharp, shooting pain in the right upper abdominal quadrant; this was accompanied by chills, sweats and fever. She became jaundiced in twenty-four hours and has remained so.

On physical examination there was marked icterus of the skin and sclerae. The abdominal findings essentially negative, except for slight tenderness below the umbilicus on the right; liver showed no signs of enlargement.

Patient was afebrile while under observation; she had no subjective symptoms. After two weeks the icterus disappeared. She refused operation.

Clinical Pathology: Urine showed the presence of bile. Urobilinogen was increased. Blood: findings normal. Stools showed no abnormalities.

Galactose, 1.25 gm. Phthalein, 14 per cent.

Case 31.—Diagnosis: Carcinoma of head of pancreas involving common duct.

This patient had always drunk one or two glasses of whisky or one or two "beers" a day. He had had no previous illness. About two weeks before admission he first noted that his urine was highly colored, his stools became clay-colored and he became jaundiced. These conditions persisted. He experienced slight pain in the region of the crest of the ilium on the right.

His skin was icteroid; he was poorly nourished, the abdomen full, several distended superficial abdominal veins. The liver markedly enlarged, the dulness beginning in the fifth interspace and extending 9.5 cm. below the costal margin in the right parasternal line. The edge rounded, the surface slightly uneven. A mass felt in the region of the gall-bladder.

Patient's stay in the hospital was uneventful; an exploratory laparotomy was advised.

Clinical Pathology: Urine contains much bile. Blood: findings essentially normal. Stools contain no bile.

Galactose, 0.69 gm.

Case 32.—Diagnosis: Carcinoma of head of the pancreas.

This patient was 52 years of age. He used liquor in excess for a long period of time. Following a fall a few days before admission he complained of pain in lower abdomen and in epigastrium. Simultaneously he became jaundiced.

On physical examination his skin was icteroid, his sclerae yellow. His liver flatness was greatly increased. A tumor was felt in the region of the gall-bladder.

Patient's condition became markedly worse. He developed cholemic symptoms and died four weeks after admission.

Necropsy showed a carcinoma involving the head of the pancreas and duodenum. The bile and pancreatic ducts were greatly distended; the liver was large, showing some cirrhosis and metastatic nodules.

Clinical Pathology: Urine contained much bile. Wassermann reaction was positive. Stool contained no bile. Galactose, 0.17 gm.

Case 33.—Diagnosis: General carcinomatosis.

This patient was operated on eighteen months ago for "cancer" of the breast. A scirrhous carcinoma was removed. Since the operation she lost much weight, had recurrent epistaxes, marked tinnitus, and malaise.

Patient markedly emaciated and very pale. The superficial lymph-nodes were enlarged; there were questionable areas of consolidation in the lungs. There was abdominal distention, with evidence of fluid in the abdominal cavity, of an enlarged liver, of a massive tumor in the region of the spleen. Patient was observed for two months. The course of the disease was not unlike that of pernicious anemia. X-ray examination showed numerous areas in the skull and long bones suggestive of multiple carcinomata.

Clinical Pathology: Blood, repeated examinations, hemoglobin from 35 to 60 per cent.; red blood-cells 1,020,000. Urine, normal findings. No Bence Jones bodies. Stool, normal findings.

Galactose none. Phenoltetrachlorphthalein, 8 per cent.

Case 34.—Diagnosis: Cirrhosis of the liver (?); adhesive pericarditis; Pick's syndrome.

When 16 years of age patient had rheumatic fever. He had a recurrent attack twelve months ago. He had not used alcohol in excess. Two years ago he developed dyspnea which became progressively worse. There had been ascites from the onset. He had been tapped repeatedly.

Physical examination showed a poorly nourished man with cardiac enlargement, with evidences of adhesive pericarditis. There were signs of fluid in the abdomen, marked edema of both legs, and evidences of collateral circulation. After paracentesis his liver was found to be enlarged. Patient observed on the wards for two months. Except for repeated abdominal tappings his course was uneventful.

Clinical Pathology: Urine showed normal findings. Phenolsulphonephthalein test: 47 per cent. output in two hours (repeated). Blood: findings not remarkable.

First phenoltetrachlorphthalein, trace; second, 8 per cent. Galactose, 0.2 gm.

Case 35.—Diagnosis: Exophthalmic goiter.

This patient has always enjoyed excellent health. About one year ago she began to lose weight; her eyes became prominent and her hands unsteady; she perspired freely and complained of palpitation of her heart.

Of note on physical examination were the exophthalmos, the presence of a Von Graefe sign, a marked tremor of the hands, the absence of a struma, a slightly enlarged heart.

The patient had two attacks of tonsillitis while under observation. Later a partial thyroidectomy was performed with relief.

Clinical Pathology: Urinary findings, normal. Blood: a moderate lymphocytosis.

Galactose, 2.5 gm. Phenoltetrachlorphthalein, 30 per cent.

Case 36.—Diagnosis: Chronic interstitial nephritis.

This patient had frequent attacks of tonsillitis: "dysentery" on three occasions while in the Philippines. Seven months ago his condition was diagnosed as nephritis at the Massachusetts General Hospital. His chief complaints were headache and failing vision. There had been some frequency of micturition and polyuria.

Patient was observed some six months and finally died in uremia. Clinically he represented a classical picture of chronic nephropathy. Blood-pressure 235. Marked albuminuric retinitis.

Clinical Pathology: Urine: specific gravity 1.010. The sediment showed numerous casts and a large trace of albumin. Phenolsulphonephthalein output 20 per cent. in two hours. Feces contained much blood during last few days of life.

The phenoltetrachlorphthalein was given while patient was in uremia. A trace was recovered in the stools.

Necropsy showed a slight degree of central necrosis.

Case 37.—Diagnosis: Peritonitis (tuberculous).

The previous history of this case unimportant. The patient complained of enlargement of her abdomen.

The physical examination showed a rather poorly nourished woman, abdomen markedly distended, signs of fluid in the abdominal cavity, no signs of collateral circulation.

On three occasions, after abdominal paracentesis, indefinite nodular masses were felt in the epigastric region. The liver was not enlarged. Pelvic examination showed negative findings. Patient was observed three weeks; her course was uneventful. After six weeks she again returned for paracentesis. At this time the masses in the epigastric region were somewhat larger than formerly. She had lost little weight. Patient was under observation about six months. She died at her home; necropsy not performed.

Clinical Pathology: Ascitic fluid: characteristics of an exudate, specific gravity, 1.021; 2.1 per cent. albumin; a lymphocytosis. Guinea-pigs injected with the fluid showed no evidence of tuberculosis. Stool, urine and blood showed normal findings.

Phenoltetrachlorphthalein, 32 per cent.

Case 38.—Diagnosis: Chronic cardiac valvular disease.

This patient had a severe attack of rheumatic fever at the age of 14. In other particulars her past and personal histories unimportant. She had had cardiac symptoms for the past twelve years and repeated (six or eight) attacks of broken compensation.

Her skin had an icteroid tinge; the sclera were similarly pigmented. The heart was enlarged and there were signs of a mitral stenosis and regurgitation. The blood-pressure was slightly elevated. The abdomen distended but no evidences of fluid. The liver dulness began at the level of the fifth rib and the edge felt 9 cm. below the costal margin in the right mammary line. At times it pulsated; there was edema of the extremities.

Clinical Pathology: Urine contained no bile but a large trace of albumin. The findings were essentially normal otherwise.

Phenoltetrachlorphthalein output, 27 per cent. (test given during period of decompensation).

Case 39.—Diagnosis: Tuberculosis (chronic, pulmonary); tuberculous peritonitis; nephritis, chronic; amyloid (?); amyloid liver (?).

Patient's past history unimportant. He had been a moderate user of alcohol. Three months ago he began to be dyspneic; he then developed edema of his legs and a slight cough with profuse expectoration.

Physical examination showed definite pulmonary involvement. The abdomen showed evidence of fluid; the liver was not enlarged. There was edema of the extremities.

Patient was under observation thirty-nine days. His course was febrile; he was discharged unimproved. During his stay in the hospital he developed a bloody diarrhea, otherwise his condition was uneventful.

Clinical Pathology: Sputum showed tubercle bacilli. Urine: average of 1.030 specific gravity; always contained a trace of albumin (from 1 to 3 per cent.); many hyaline and granular casts. Phenolsulphonephthalein output in two hours was 50 per cent. Stools contained much blood; no tubercle bacilli. Blood showed normal findings.

Phenoltetrachlorphthalein, 14 per cent.

Case 40. — Diagnosis: Carcinoma of stomach (?) with metastases; cholelithiasis (?).

This patient had always taken one or two glasses of beer a day, also whisky occasionally. About twenty-six years ago she had gastric symptoms which were ascribed to a gastric ulcer, also occasional attacks of indigestion since, characterized by burning pain in the stomach. For the past nine months patient had severe cramp-like pain originating about the umbilicus and radiating toward the left axilla. She had repeated attacks lasting three or four days, with no jaundice; ten days ago she had a very severe attack, with much vomiting, diarrhea, fever. Following the attack she had marked tenderness over the right hypochrondrium. There had been considerable loss of weight.

The liver was found to be enlarged, tender, with questionable nodules over the lower surface. An indefinite mass made out, apparently connected with the liver, extending to the umbilicus.

Patient was observed twenty-one days; the acute attack subsided. She refused operation. Her general condition was unimproved. X-ray examination showed a typical hour-glass stomach.

Clinical Pathology: Gastric examination showed a marked anacidity. Gastric infiltration and x-ray findings showed an hour-glass pattern. Stool and urine examinations showed nothing abnormal. Wassermann reaction was negative. Blood: negative findings.

First phenoltetrachlorphthalein, 10 per cent.; second, 20 per cent.

Case 41.—Diagnosis: Tuberculous peritonitis.

This patient's personal history unimportant. He enjoyed excellent health up to two months ago, when his abdomen began to swell. He had been constipated.

On physical examination he was found to be very poorly nourished. There were questionable signs of pulmonary involvement. The abdomen manifested signs of fluid in the peritoneal cavity. After paracentesis the liver was found to extend 7 cm. below the costal margin. It was smooth except for a nodular elevation below the xyphoid process.

Below the angle of the left scapula there was an abscess formation. Pus and tissue recovered from this showed tuberculous lesions. Guinea-pigs injected with it developed tuberculous lesions. Patient's course was febrile; he became progressively worse and died about four months after onset of the disease. Necropsy not obtained.

Clinical Pathology: Otherwise unimportant.

Galactose: 0.6 gm.

TABLE 1.—NORMALS

Case No. *	Hospital No.	Diagnosis	Galactose gm.	Phthalein † Feces (%)	Remarks
1	M. 394	Cardiac chr. valvular	....	44	Lesion well compensated when test was given.
2	M. 544	Cardiac	....	40	Lesion well compensated when test was made.
3	M. 460	Cardiac	....	41	Compensated when test was made.
4	Huntington Hosp.	Carcinoma of breast	....	32	
5	M. 755	Syphilis	0.0	32	Autopsy.
6	M. 808	Von Recklinghausen dis.	....	34	
7	M. 818	Pleurisy	....	36	
8	M. 30	Spastic paraplegia	1.5	..	

* The salient features of each case in this and the following tables will be found in the accompanying protocols.

† None was found in the urine in any case.

TABLE 2.—CATARRHAL JAUNDICE (ICTERUS CATARRHALIS)

Case No.	Hospital No.	Diagnosis	Galactose gm.	Phthalein Per Cent.	Remarks
9	M. 785	Icterus catarrhalis (uncomplicated)	2.6	1— 0 feces 6 urine 2—10 feces 0 urine 3—20 feces 0 urine	Galactose given day of admission. First phthalein two days after admission. Second phthalein ten days after admission. Third phthalein given three weeks after admission.
10	M. 158	Icterus catarrhalis; cirrhosis of liver.	1st—0.18 2d —1.7	† feces † urine	Galactose given on first admission (see history). Second galactose given in later periods of disease. Stools clay-colored at time phthalein was given.
11	M. 423	Icterus catarrhalis; cirrhosis of liver.	4.3	Not given	
12	M. 585	Icterus catarrhalis	Not given	10	Stools clay-colored at time of test.
13	M. 147	Icterus catarrhalis	3.9	0	Jaundice totally disappeared while in hospital.

† Trace.

TABLE 3.—Cirrhosis of Liver

Case No.	Hospital No.	Diagnosis	Galactose gm.	Phthalein Per Cent.	Remarks
14	M. 757	Cirrhosis	0.0	1—20 feces 0 urine 2—20 feces 0 urine	Excellent collections.
15	M. 316	Cirrhosis, nephritis (chronic)	0.4	1—22 feces 0 urine 2—20 feces 0 urine	Excellent collections.
16	M. 769	Cirrhosis	0.9	1— 7 feces 0 urine 2—19 feces 0 urine	Collections of first phthalein test not satisfactory. Necropsy.
17	M. 442	Cirrhosis	0.4	40 feces † ?	
18	M. 678	Cirrhosis, nephritis (chronic)	*	1—20 feces 0 urine 2—25 feces 0 urine	
19	M. 489	Cirrhosis, nephritis (chronic)	0.7	Test performed. Collections unsatisfactory.	
20	M. 171	Cirrhosis	0.94	....	This case may also represent a condition of icterus catarrhalis in the last period of the disease.
21	M. 816	Cirrhosis	0.0	1—36 feces 0 urine	Excellent collections.
22	M. 1030	Cirrhosis	2.21	36 feces 0 urine	
23	M. 36	Cirrhosis	‡	45 feces 0 urine	Patient vomited after taking galactose.
24	Huntington Hosp.	Cirrhosis (syphilitic)	1.4	45 feces 0 urine	
25	M. 1151	Haemachromatosis, cirrhosis of liver	0.6	†	
26	M. 1168	Cirrhosis	†	36 feces † urine	

* Not given. ‡ Unsatisfactory. † Trace. † ? Slight trace in urine.

TABLE 4.—CASES WITH JAUNDICE DUE TO OBSTRUCTION OF COMMON OR CYSTIC DUCT

Case No.	Hospital No.	Diagnosis	Galactose gm.	Phthalein Per Cent.	Remarks
27	M. 5	Cholelithiasis; carcinoma of head of pancreas	0.0	0 feces † urine	At time of test urine contained much bile, the stools were clay-colored.
28	M. 634	Carcinoma of head of pancreas	0.0	1— 0 feces † urine 2— 0 feces 3—29 feces 0 urine	First phthalein test was given before operation, with bile in urine. Second given three days after operation. Third three weeks after operation. Jaundice practically disappeared. Stools normal color.
29	M. 281	Carcinoma of head of pancreas	0.0	....	Necropsy. Diagnosis confirmed.
30	M. 506	Cholelithiasis	1.25	14 feces 0 urine	Patient was slightly jaundiced at time of test.
31	M. 1028	Carcinoma of head of pancreas.	0.69	....	
32	M. 310	Carcinoma of head of pancreas; cirrhosis of liver.	0.37	....	Necropsy. Diagnosis confirmed.

TABLE 5.—MISCELLANEOUS

Case No.	Hospital No.	Diagnosis	Galactose gm.	Phthalein Per Cent.	Remarks
33 *	M. 602	Multiple carcinomatosis	0.0	8 feces † ? urine	Excellent collections.
34	M. 710	Polyserositis	0.2	1— † feces 0 urine 2— 8 feces 0 urine	Excellent collections.
35	M. 405	Exophthalmic goiter	2.5	30 feces 0 urine	
36	M. 146	Nephritis (chronic); uremia	....	† feces	Test made when patient was in uremia, two days before death. The catharsis for the first eighteen hours excellent. Much blood in stools.
37	M. 687	Peritonitis (tuberculous)	0.29	32 feces 0 urine	
38	M. 513	Cardiac chronic valvular	....	37 feces † ? urine	Lesion poorly compensated.
39	M. 536	Tuberculosis (general)	....	14 feces	
40	S. 592	Carcinoma of stomach, metastases in liver; cholelithiasis.	....	1—10 feces 2—20 feces 0 urine	
41	M. 152	Peritonitis (tubercular)	0.6		

† Trace † ? Slight trace.

STUDY XXII: THE EFFECT OF THEOBROMIN SODIUM SALICYLATE IN ACUTE EXPERIMENTAL NEPHRITIS, AS MEASURED BY THE EXCRETION OF PHENOLSULPHONEPHTHALEIN *

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The therapeutic efficacy of diuretic drugs on patients with acute nephritis has been discussed by most writers in their description of methods of management of acute nephritis. The views expressed have been based largely on clinical observation, and there has been relatively little experimental evidence[1] accumulated in support of the claims made for the usefulness, within certain limitations, of diuretic drugs in acute nephritis. Inasmuch as clinical observation of acute nephritis is much hampered by the infrequency of uncomplicated cases and by the difficulties in determining whether any two patients actually present comparable renal lesions, it has seemed desirable to have more observations under experimental conditions in which some of the factors are controllable in the hope that gradually data may be accumulated as a basis for a more efficient method of treating acute nephritis. The following experiments are reported for this reason. Though inconclusive in many respects, it is hoped that they may throw some slight light on the problem and at least be helpful to other investigators by showing what has happened to these animals under the conditions of the experiment.

The experiments here reported concern themselves with one diuretic (theobromin sodium salicylate or diuretin*) used in one form of

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* Submitted for publication May 7, 1914.

* From the Laboratory of the Theory and Practice of Physic, Harvard Medical School, and the Medical Clinic of the Peter Bent Brigham Hospital, Boston.

* This is one of a series of studies on experimental cardiorenal disease. Study I, Smith: Boston Med. and Surg. Jour., 1908, clviii, 696; Study II, Christian: Boston Med. and Surg. Jour., 1908, clix, 8; Study III, Christian: Jour. Am. Med. Assn., 1909, liii, 1792; Studies IV-XV, Christian, Smith and Walker: The Archives Int. Med., 1911, viii, 468-551; Study XVI, Christian and O'Hare: The Archives Int. Med., 1913, xi, 517; Studies XVII and XVIII, O'Hare: The Arhives Int. Med., 1913, xii, 49, 61; Study XIX, Christian and O'Hare: Jour. Med. Research, 1913, xxviii, 227; Study XX, Walker and Dawson: The Archives Int. Med., 1913, xii, 171; Study XXI, Fitz: The Archives Int. Med., 1914, xiii, 945.

[1] See Study XXI by R. Fitz (The Archives Int. Med., 1914, xiii, 945) for a report of the immediate effects of theobromin sodium salicylate and theocin in experimental nephritis and for a review of other experimental work on the effects of diuretics.

* The preparation used throughout these experiments was made by Knoll & Co., and purchased from retail druggists under the name "diuretin."

acute nephritis (that produced by uranium nitrate) in a single laboratory animal (rabbit). Forty-nine animals were utilized and they were kept under similar conditions in metabolism cages on a diet of carrots, oats and water. The amount of each food taken by the rabbit varied with the animal's appetite, but all were measured. The urine was collected and measured in seventy-two- or twenty-four-hour periods. The uranium nitrate was given subcutaneously in doses of 3.5, 1.1, 0.5 and 0.25 mg. per kilogram of body weight, respectively, to different groups of animals. Forty-eight hours later theobromin sodium salicylate was

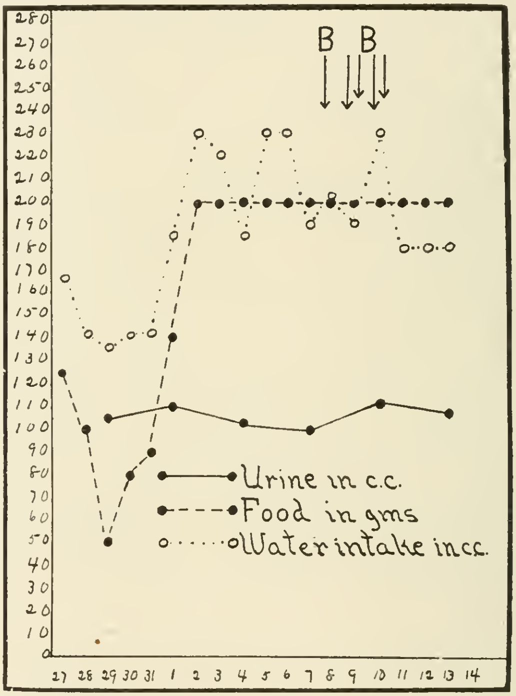

Chart 1.—Rabbit 86. *B,* theobromin sodium salicylate, 14 mg. per kilogram of body weight.*

given subcutaneously to a portion of these animals in a dose (14 mg. per kilogram of body weight) comparable to the therapeutic dose for man, and this was repeated in most cases twice daily for several days. The phenolsulphonephthalein excretion was determined at intervals; the phenolsulphonephthalein was given intramuscularly in a dose of 6 mg., and the urine for quantitation of the phenolsulphonephthalein excretion was obtained by catheterization with thorough washing out of the bladder; quantitation was carried out by the usual colorimetric method.

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* Numerals along bottom of charts indicate days of the month.

The rabbits were studied in small groups at different periods between May, 1913, and April, 1914, and these groups were obtained from several dealers; consequently, in order to reduce the variants to a minimum, only animals within the several groups have been compared.

As a preliminary control, normal rabbits were given theobromin sodium salicylate in the way described. The result was practically nil, as shown by the chart[2] of Rabbit 86 (Chart 1). Other animals showed the same thing.

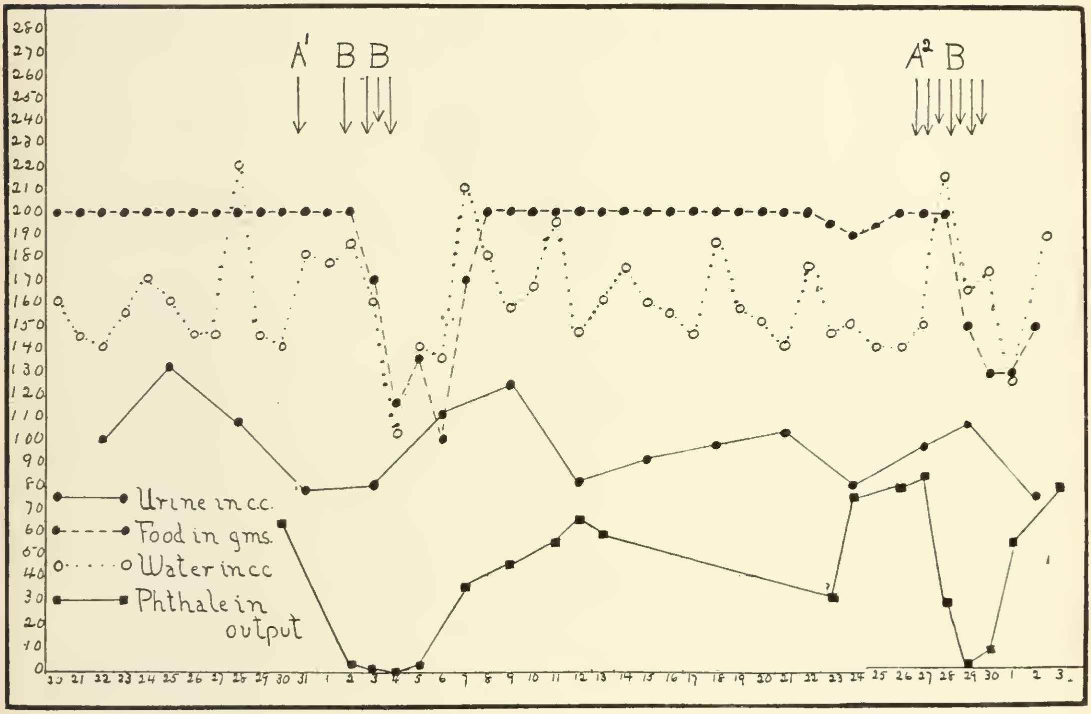

Chart 2.—Rabbit 686. Period of phenolsulphonephthaein excretion, one hour, 33 minutes. *A*¹, uranium nitrate, 3.5 mg. per kilogram of body weight. *A*², uranium nitrate, 1.1 mg. per kilogram. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

In the first series 3.5 mg. of uranium nitrate per kilogram of body weight was given to six animals (Rabbits 686, 687, 688, 689, 691 and 692) with results as shown in illustrative charts of a few rabbits[3] (Charts 2 and 3). This amount of uranium nitrate produces a severe

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[2] In all charts "water intake" includes water content of carrots calculated as 88 per cent. of total weight of carrots eaten.

[3] Owing to expense of reproduction only a few of the charts which were made of each rabbit have been printed.

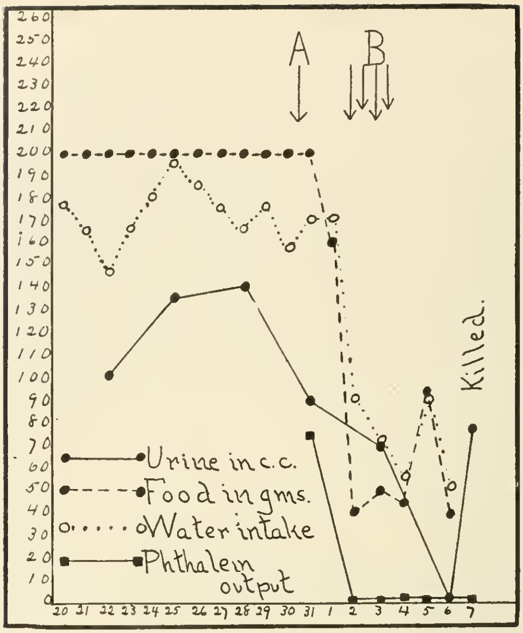

Chart 3.—Rabbit 688. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate, 3.5 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

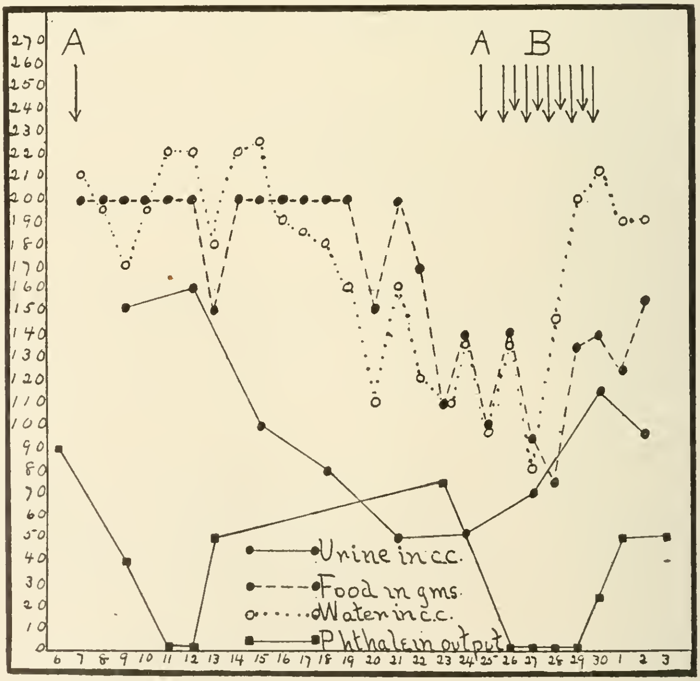

Chart 4.—Rabbit 701. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate, 1.1 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

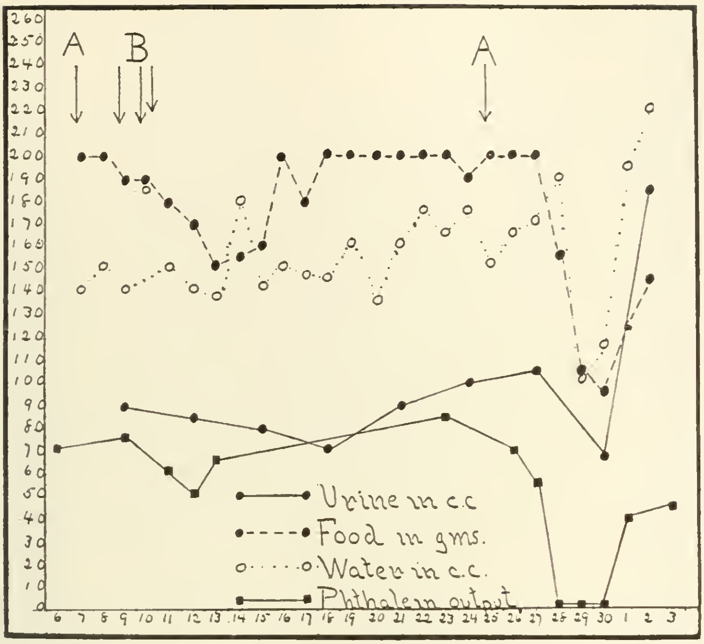

Chart 5.—Rabbit 702. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium nitrate 1.1 per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

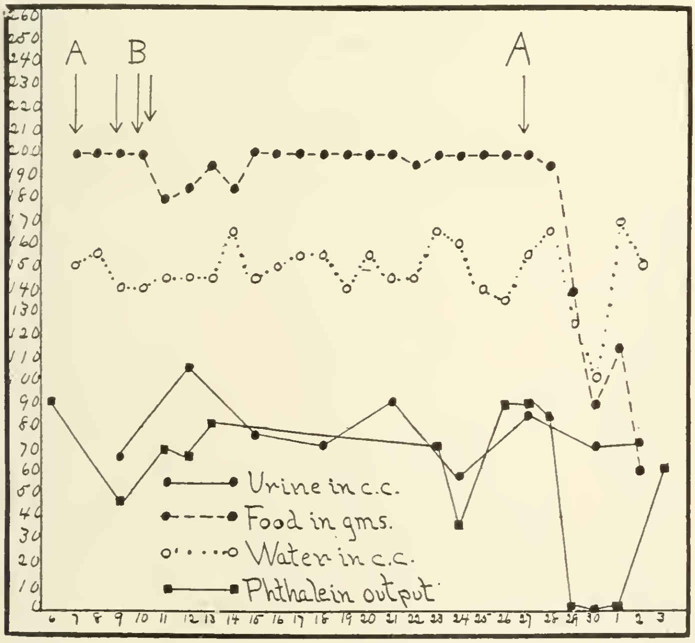

Chart 6.—Rabbit 705. Period of phenolsulphonephthalein excretion, one hour, thirty minutes. *A*, uranium nitrate, 1.1 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

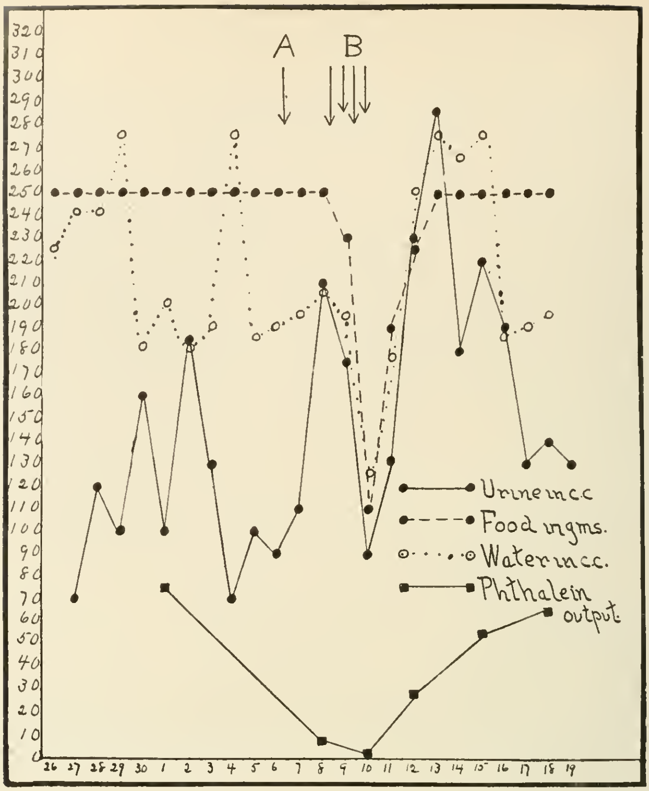

Chart 7.—Rabbit 816. Period of phenolsulphonephthalein excretion, one hour. *A,* uranium nitrate, 0.5 mg. per kilogram of body weight. *B,* theobromin sodium salicylate, 14 mg. per kilogram.

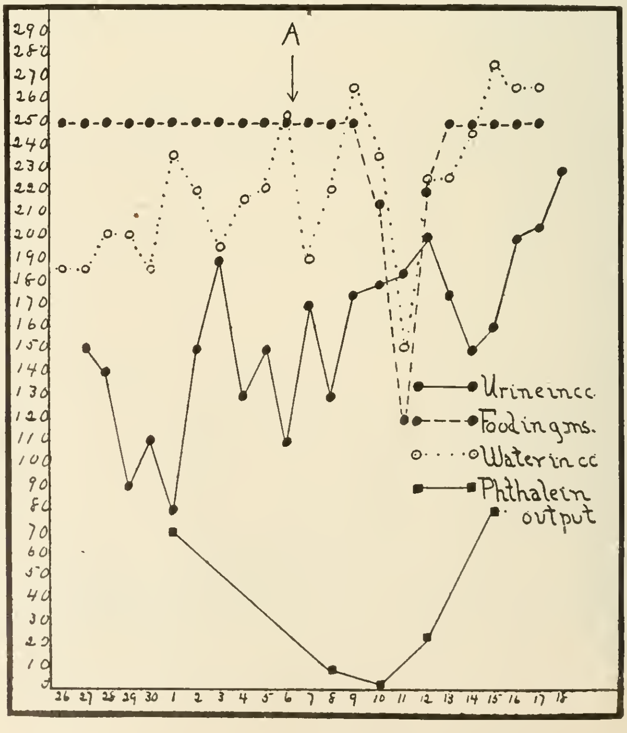

Chart 8.—Rabbit 819. Period of phenolsulphonephthalein excretion, one hour. *A,* uranium nitrate, 0.5 mg. per kilogram of body weight.

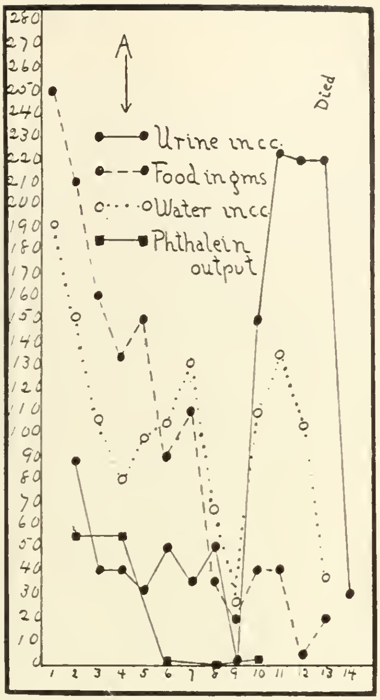

Chart 9.—Rabbit 790. Period of phenolsulphonephthalein excretion, one hour. *A,* uranium nitrate, 0.5 mg. per kilogram of body weight.

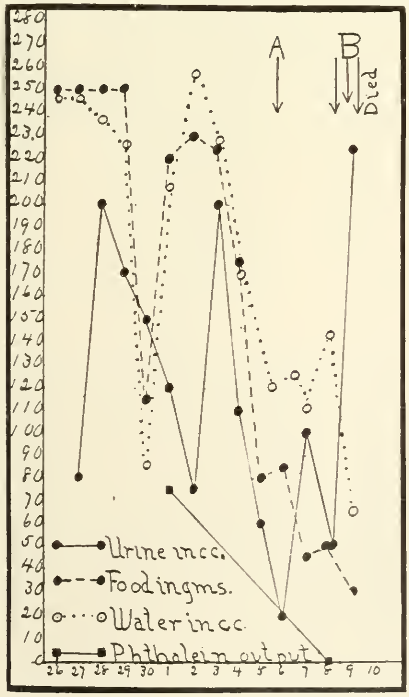

Chart 10.—Rabbit 815. Period of phenolsulphonephthalein excretion, one hour. *A,* uranium nitrate, 0.5 mg. per kilogram of body weight. *B,* theobromin sodium salicylate, 14 mg. per kilogram.

usually fatal acute nephritis (see Christian and O'Hare[4] for average duration of life and lesion produced with and without theobromin sodium salicylate), and in these six rabbits all died in a short time, except one (Rabbit 686, Chart 2), to which theobromin sodium salicylate was given and which subsequently survived a smaller dose of uranium nitrate. In the five animals which died, those with and without theobromin sodium salicylate showed the same curve of phenolsulphonephthalein excretion, indicating a marked disturbance of function uninfluenced by theobromin sodium salicyate, though in one rabbit (688, Chart 3) a diuresis immediately preceded death. Whether or not theobromin sodium salicylate was responsible for the survival of

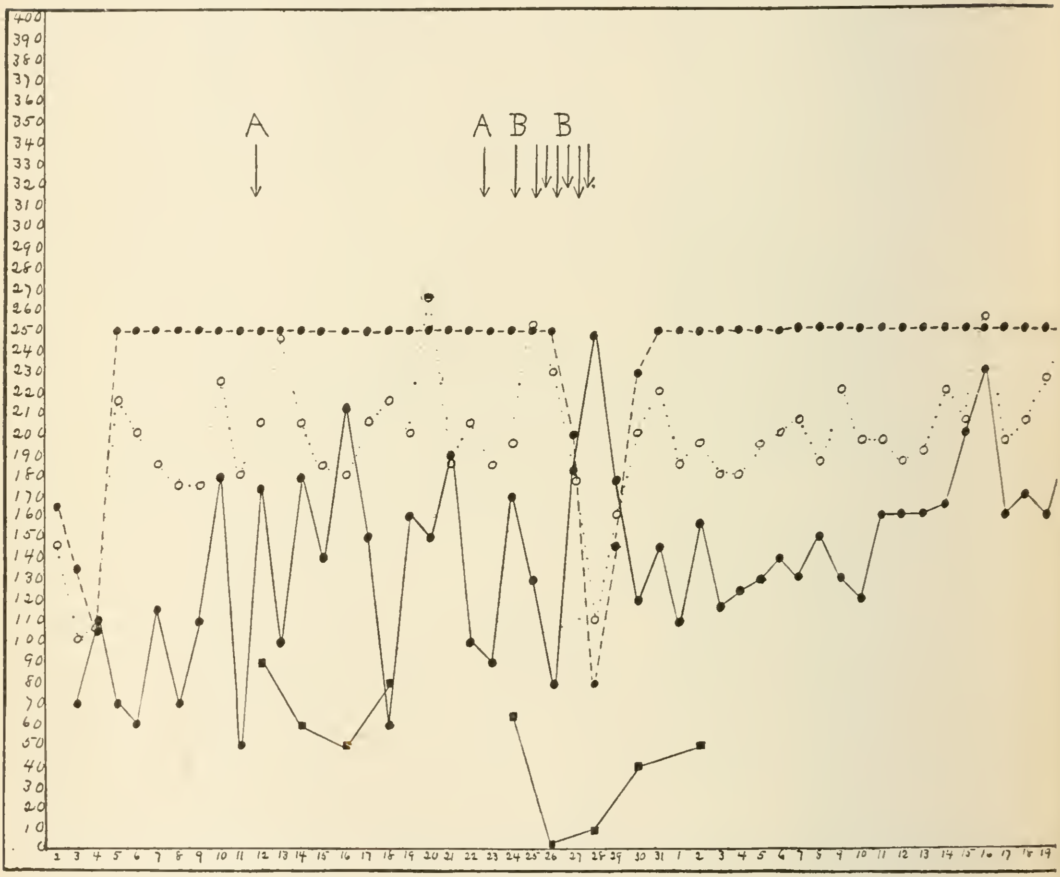

Chart 11.—Rabbit 849. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium

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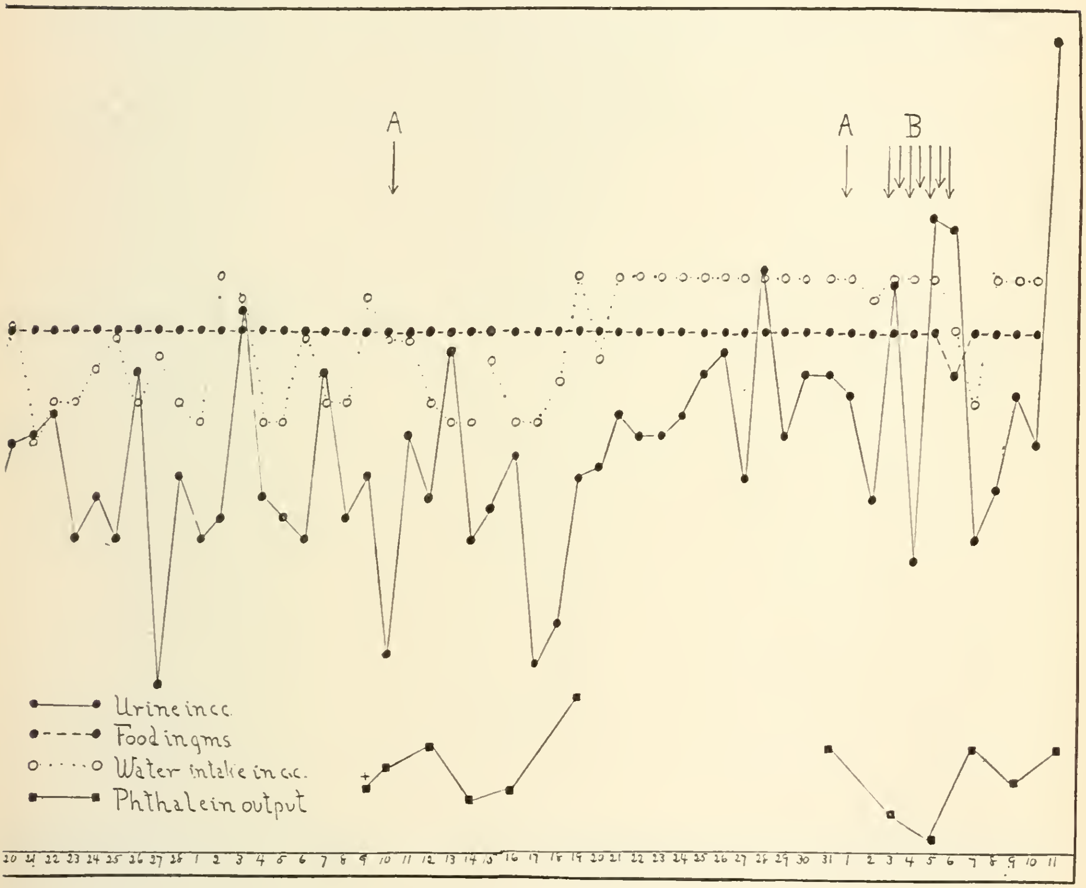
[4] Christian, Henry A., and O'Hare, James P.: A Study of the Therapeutic Value of a Diuretic (Theobromin Sodium Salicylate or Diuretin) in Acute Experimental Nephritis (Study XVI), The Archives Int. Med., 1913, xi, 517.

Rabbit 686 cannot be determined. It is known that rabbits vary in their susceptibility to uranium nitrate, and this may represent an unusual degree of natural resistance. This was observed in several rabbits of a previous series (Christian and O'Hare[4]), although in most of the rabbits in that study with severe nephritis theobromin sodium salicylate shortened life.



nitrate, 0.25 mg. per kilogram of body weight. *B,* theobromin sodium salicylate, 14 mg. per kilogram.

In the second (Rabbits 701, 702, 703, 704 and 705), third (Rabbits 707-708), fourth (Rabbits 709-716) and fifth (Rabbits 744-752) series of animals 1.1 mg. of uranium nitrate per kilogram of body weight was given, with results as shown in illustrative charts of some of the rabbits (Charts 4, 5 and 6). In two series the urine was collected at seventy-two-hour intervals, and in two at twenty-four-hour intervals. Of the second series, Rabbits 701, 702 and 705 received uranium nitrate twice nine days apart, once followed by theobromin sodium salicylate. These rabbits (701, 702 and 705, Charts 4, 5 and 6) suggest a slight beneficial effect of theobromin sodium salicylate on renal function as measured by phenolsulphonephthalein excretion, but this effect is apparent rather than real when allowance is made for a probably more intense action of the second dose of uranium in an animal that a short time previously has had an acute renal lesion produced by the same dose of uranium nitrate.

If the curve of phenolsulphonephthalein excretion in all of the twenty-four animals of Series 2 to 5 is studied, it is seen that in a number theobromin sodium salicylate appears to have a slight beneficial action as expressed either by a slowed fall or a more prompt return to the normal phenolsulphonephthalein output. This is offset, however, by the fact that of the six rabbits that died, four had been given theobromin sodium salicylate. The amount of uranium given these rabbits, 1.1 mg. per kilogram of body weight, caused a severe nephritis in most, as shown by the 25 per cent. mortality, the marked loss of appetite, the decreased urine output, and the fall in phenolsulphonephthalein output to mere traces. In severe acute nephritis as shown by these series, as

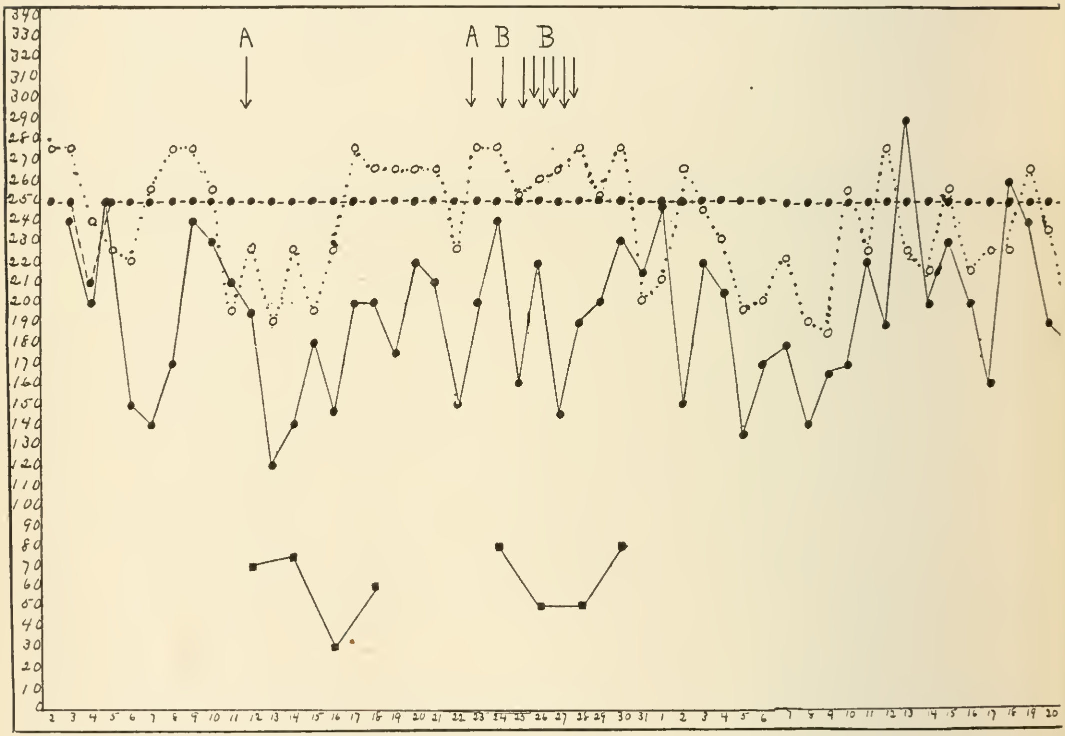
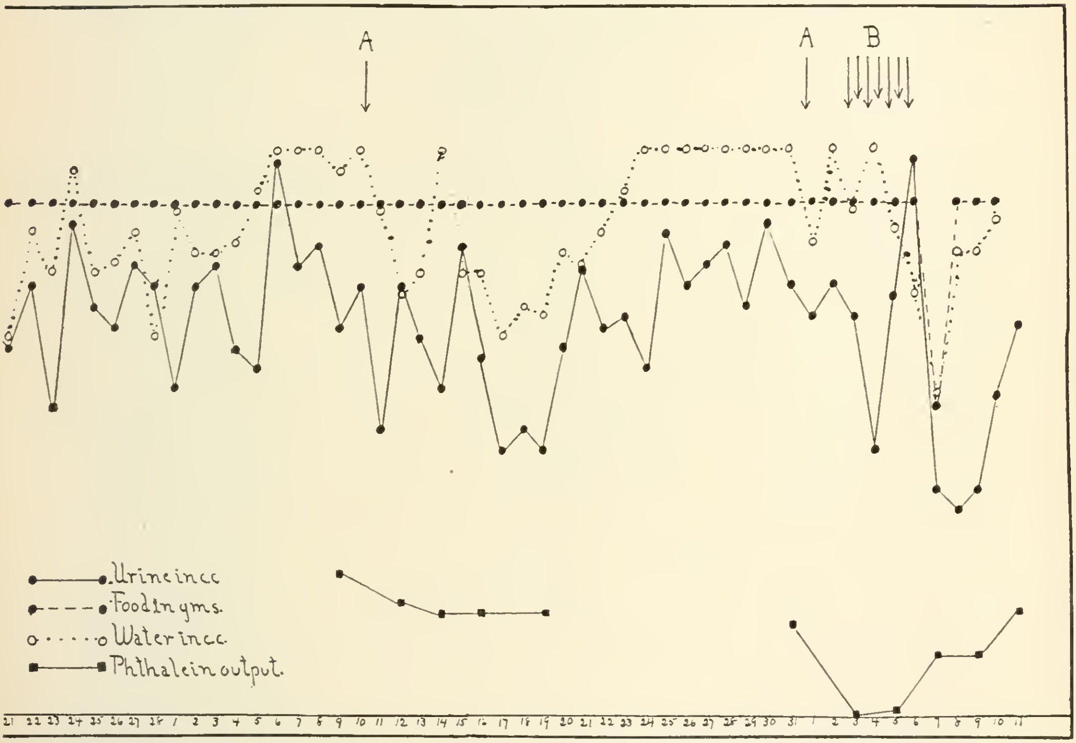


Chart 12.—Rabbit 850. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium

well as by Series 1, it does not appear that theobromin sodium salicylate exerts any evident beneficial action on renal function as measured by phenolsulphonephthalein excretion; occasionally it seems to do good, but on the other hand, occasionally it seems to do harm. Consequently, it may be said that its use in rabbits with so severe an acute nephritis is a questionable therapeutic measure.

Examination of the charts shows that diuresis, though common, is not constant under the conditions of these experiments, and that it may occur a few days after the uranium is given, whether or not a diuretic is used. What is quite striking is that urine output and renal function as measured by phenolsulphonephthalein excretion do not necessarily run parallel, and sometimes animals with marked diuresis die.* In these animals phenolsulphonephthalein output is distinctly a better prognostic sign than urine output.



nitrate, 0.25 mg. per kilogram of body weight. *B,* theobromin salicylate, 14 mg. per kilogram.

In Series 6 (Rabbits 789, 790, 791) and 7 (Rabbits 815-819) still smaller doses of uranium nitrate were used, 0.5 mg. per kilogram of body weight (for results see Charts 7, 8, 9 and 10 of illustrative animals). Two rabbits, 790 and 815 (Charts 9 and 10), one with and one without theobromin sodium salicylate, show a marked diuresis preceding death. In these series the charts show a slightly more evident beneficial effect from theobromin sodium salicylate, though this is really too slight to be considered of much importance and might be easily a matter of coincidence rather than cause and effect. Since the lesion in most of these animals is a severe one, for three out of nine died, one having received theobromin sodium salicylate, two not, it seemed desirable to use still smaller doses of uranium nitrate.

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* None of the charts selected for printing from these series show this but it is shown in Charts 9 and 10 of the next series.

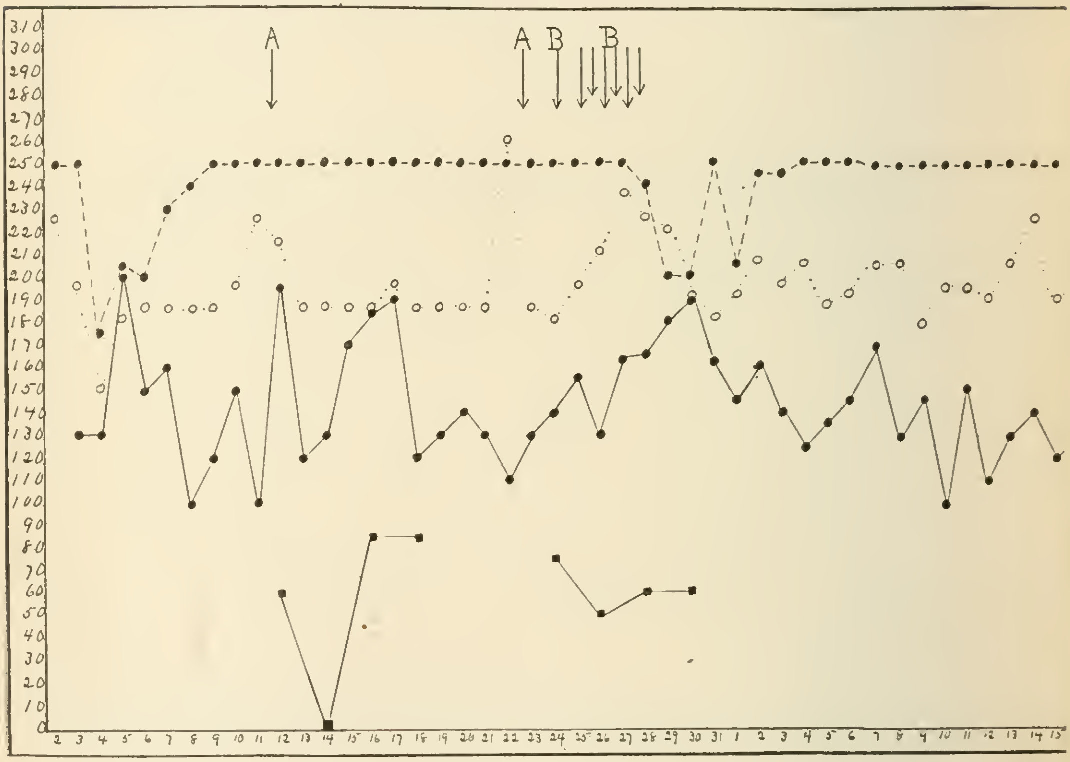
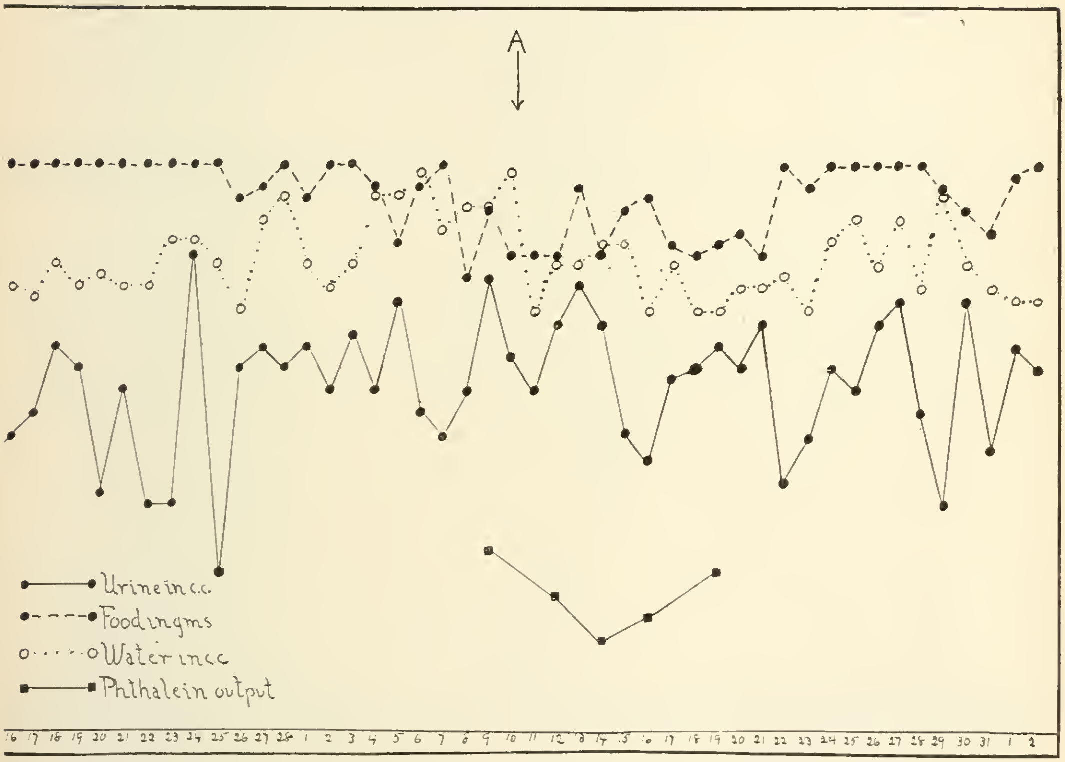


Chart 13.—Rabbit 851. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium

In the last series (Series 8, Rabbits 849-854) 0.25 mg. of uranium nitrate per kilogram of body weight was used. Inasmuch as it seemed likely that this dose would not produce any profound change in the kidney not easily recoverable from, a slightly different method was followed in that uranium was repeated after a time interval thought to be long enough for recovery. With the first dose of uranium no rabbit received theobromin sodium salicylate, and this dose was used only as a test of the rabbit's susceptibility to uranium. One rabbit, 853, died even after this small dose of uranium. This animal showed a precritical diuresis. Rabbits 849, 850, 851, 852 and 854 (Charts 11, 12, 13, 14 and 15) showed a very slight disturbance of renal function as evidenced by decreased output of phenolsulphonephthalein. That the disturbance was slight is shown by the prompt return to normal of the phenolsulphonephthalein excretion and the failure of the rabbits to have any decrease in appetite. One rabbit (852, Chart 14) showed no decrease in phenolsulphonephthalein, but a slight loss in appetite. After ten days, uranium was repeated to the five remaining rabbits, and to three of these theobromin sodium salicylate was subsequently given. In these, in general theobromin sodium salicylate appeared to decrease slightly functional disturbance as evidenced by phenolsulphonephthalein excretion, but not to have much effect as indicated by the animals' appetite. All five animals showed moderately increased diuresis subsequent to the uranium, but this diuresis was not definitely influenced by theobromin sodium salicylate.



nitrate, 0.25 mg. per kilogram of body weight. *B,* theobromin sodium salicylate, 14 mg. per kilogram.

Forty-six days following the second dose of uranium, the same animals received a third dose of uranium. To the two not receiving theobromin sodium salicylate previously this drug was given. In

Rabbits 849 (Chart 11) and 850 (Chart 12) without theobromin sodium salicylate, phenolsulphonephthalein excretion was slightly less depressed after uranium than had previously been the case when theobromin sodium salicylate had followed the uranium. In Rabbit 851 (Chart 13) the reverse was true. In Rabbit 852 (Chart 14) with theobromin sodium salicylate the curve of phenolsulphonephthalein excretion showed a delayed fall and a slightly more abrupt rise than previously without theobromin sodium salicylate, while in Rabbit 854 (Chart 15) with theobromin sodium salicylate the phenolsulphonephthalein curve showed much less of a fall than had been the case in this rabbit without theobromin sodium salicylate.

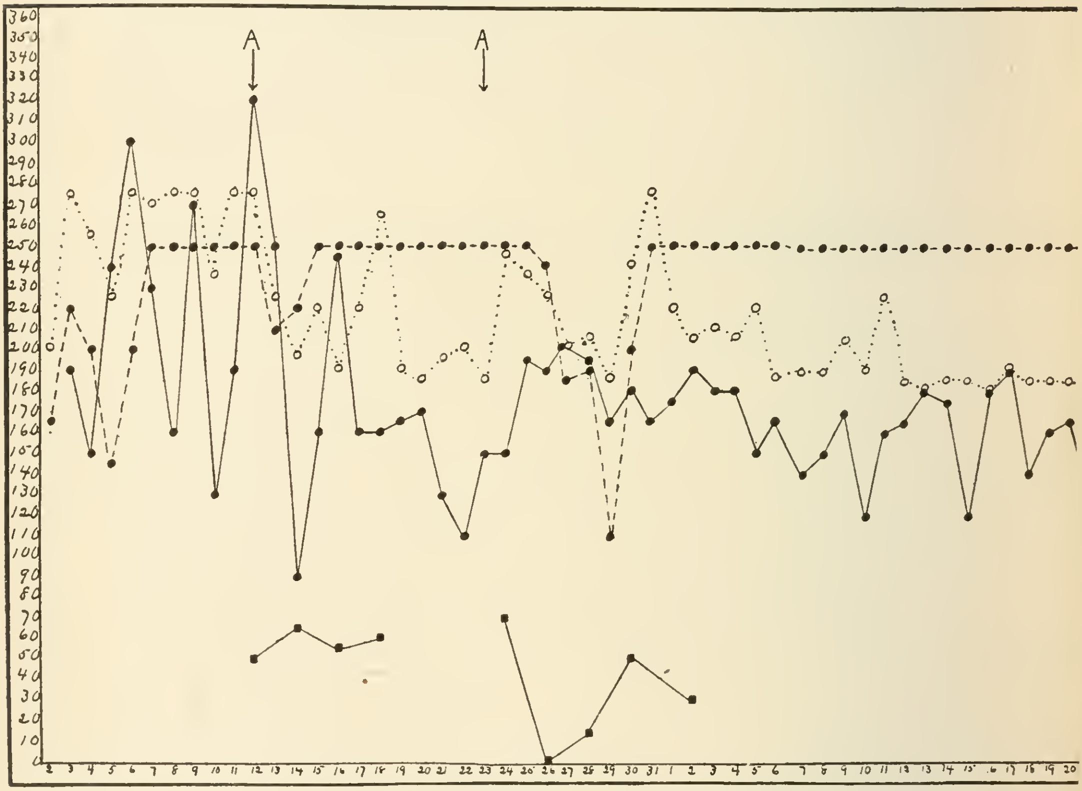
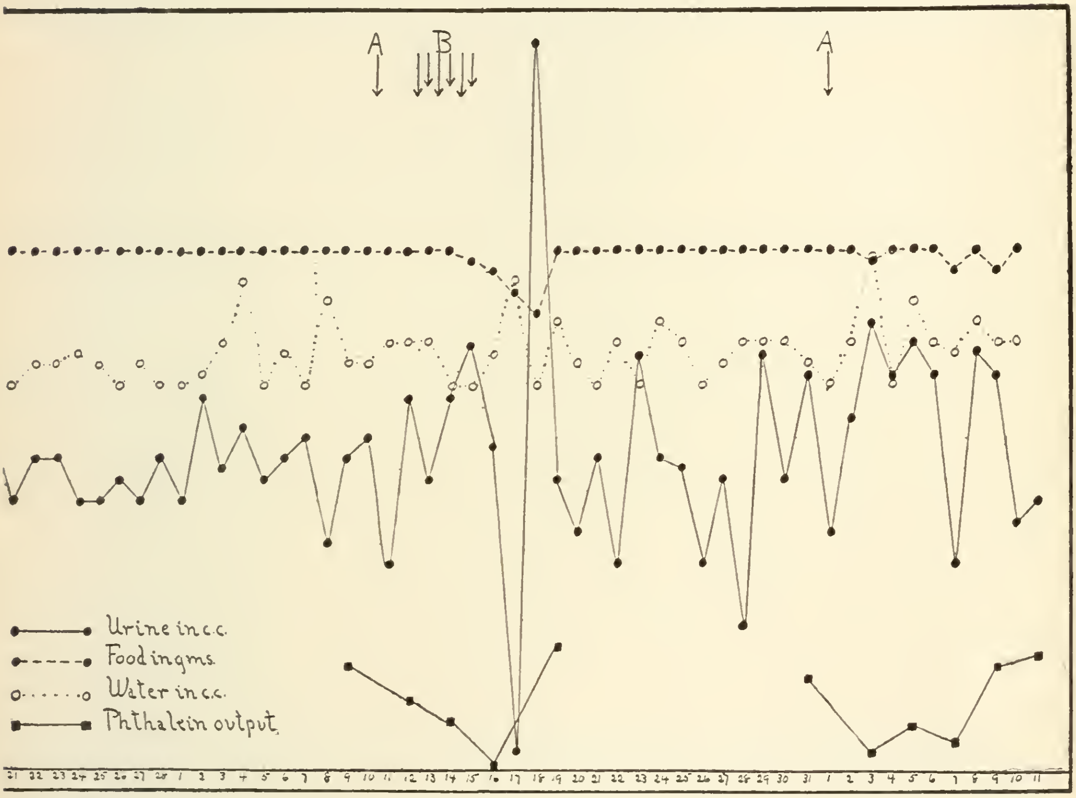


Chart 14.—Rabbit 852. Period of phenolsulphonephthalein excretion, one hour. *A*, uranium

If we compare the phenolsulphonephthalein excretion with and without theobromin sodium salicylate in these rabbits after the second and third dose of uranium, the curves taken all together show a slightly better excretion with theobromin sodium salicylate than without it. The difference, however, is slight, for it appeared in favor of theobromin sodium salicylate in but three out of five experiments, and in these the differences were quantitatively slight.

Finally, in three rabbits, 849, 850 and 852 (Charts 11, 12 and 14) a fourth injection of uranium was given twelve days following the third, and after this dose theobromin sodium salicylate was given to two animals. Here the curves were distinctly though slightly against this diuretic improving renal function.



nitrate, 0.25 mg. per kilogram of body weight. *B,* theobromin sodium salicylate, 14 mg. per kilogram.

If the last group with very slight degrees of renal disturbance is viewed as a whole and if allowance is made for some probable variation in effect from so small a dose of uranium, to say nothing of variations in results obtained at times without explainable cause in all of the observations here reported, the conclusion would seem justifiable that even in very slight degrees of acute experimental (uranium) nephritis in the rabbit there is little evidence of any beneficial action from theobromin sodium salicylate on renal function as measured by phenolsulphonephthalein excretion. It is equally true that there is little evidence of any harmful action. Rather are the results suggestive of no constant action.

SUMMARY

So far as renal function may be judged from phenolsulphonephthalein excretion in acute experimental nephritis produced by uranium in the rabbit, there is no evidence that theobromin sodium salicylate exerts any constant beneficial action. In the severer forms there is almost no evidence of beneficial action; in the very mild forms there is occasionally evidence of beneficial action, but this is too inconstant to justify the conclusion that theobromin sodium salicylate really improves renal function even in the very mild forms of acute nephritis. As other experimental evidence (Christian and O'Hare[4]) has shown the injurious action of theobromin sodium salicylate in shortening the life of rabbits with severe acute experimental (uranium) nephritis, it would seem fair to conclude that there is considerable evidence against

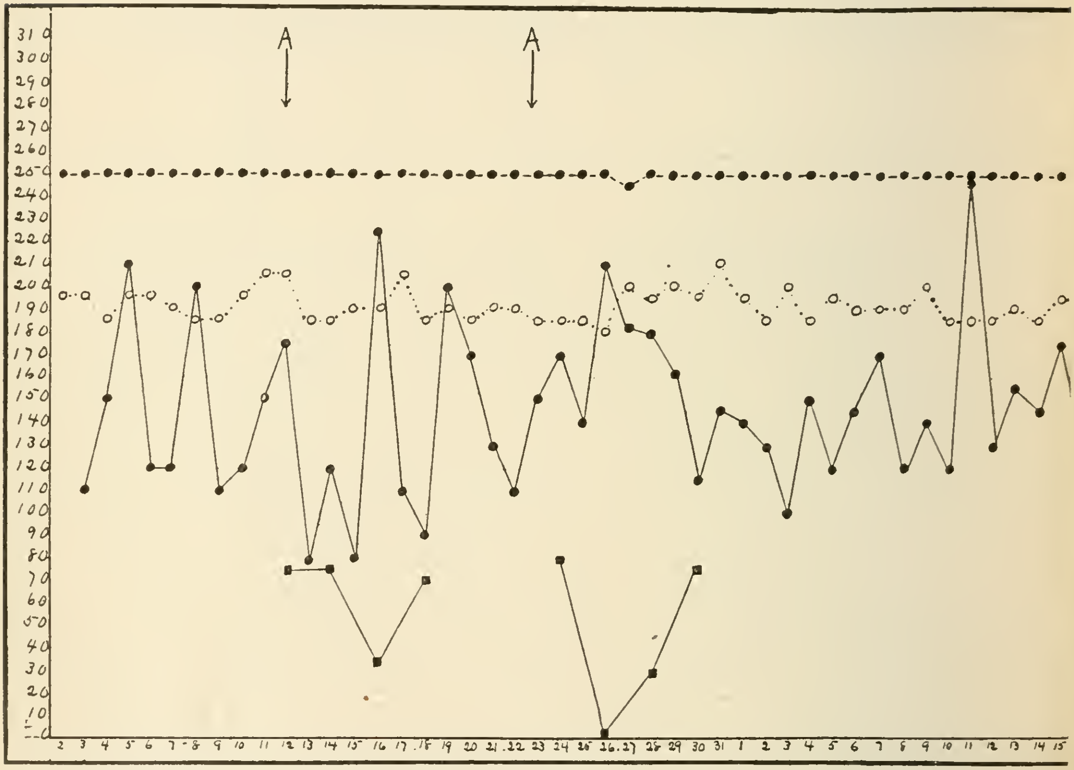
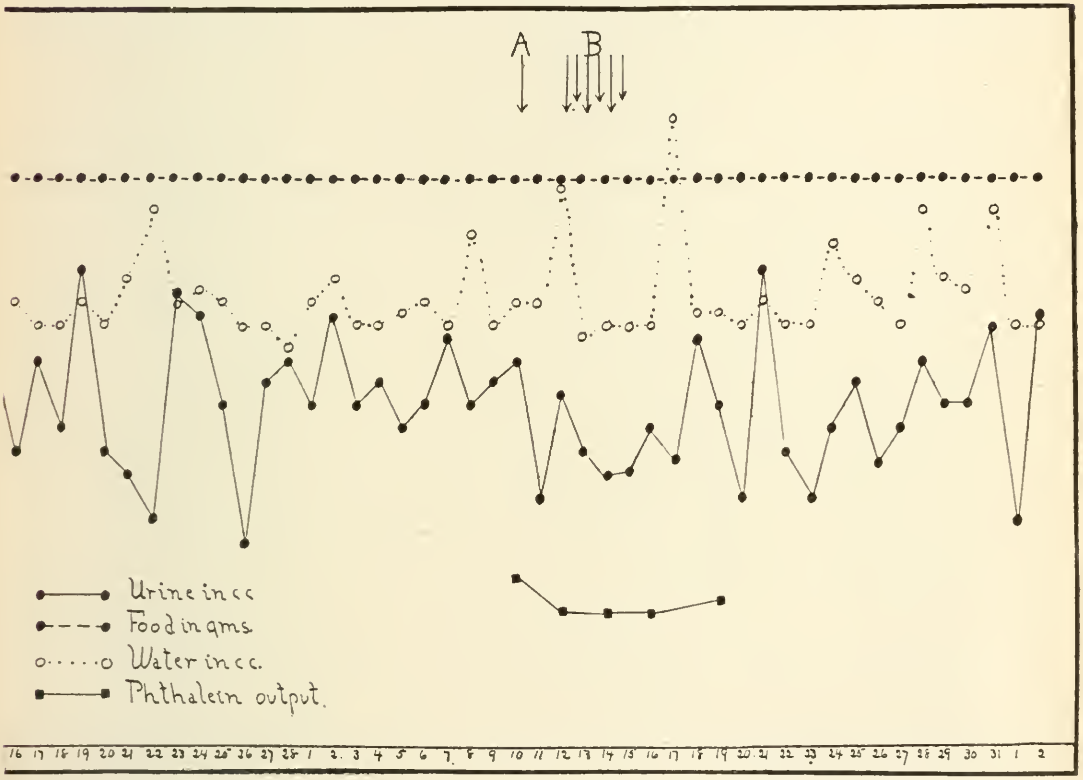


Chart 15.—Rabbit 854. Period of phenolsulphonephthalein excretion, one hour. *A,* uranium

the use of theobromin sodium salicylate in severe experimental nephritis, and very little in its favor in milder forms in which the conditions of experimentation involve observation over several days. It hardly seems necessary to add that it should be clearly kept in mind that the evidence adduced above concerns itself only with the rabbit, and the rabbit with acute nephritis of a single type, that produced by uranium, and that only one measure of renal function (phenolsulphonephthalein excretion) is being used. The results obtained in the rabbit are in accord with the common clinical teaching that diuretics are not indicated in acute nephritis in man; but they are no proof of the correctness of this teaching, for results obtained in rabbits cannot be directly read in terms of man. However, they would seem to lend some further support to the view based on clinical observation that diuretics are not indicated in acute nephritis.



nitrate, 0.25 mg. per kilogram of body weight. *B*, theobromin sodium salicylate, 14 mg. per kilogram.

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NITROGEN METABOLISM AND THE SIGNIFICANCE OF THE NON-PROTEIN NITROGEN OF THE BLOOD IN EXPERIMENTAL URANIUM NEPHRITIS *

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INTRODUCTION

It has been known for a long time that, in the course of any nephritis, there are certain periods when the urinary constituents are normal in amount as compared with the intake, and other intervals when they may be increased or diminished. To fathom the reasons for these changes in excretion seemed, until recently, an impossible task. Von Noorden[1] begged the question by characterizing the metabolism of nephritis as "bizarre and inexplicable," and many others have followed his example. Observations have been generally based on the assumption that nephritis, whether involving the glomeruli or the tubules, has only one effect on function: a diminished excretory activity of the part. Such an assumption lacks proof and is not in accord with the clinical conditions as they are generally acknowledged to exist. During the past few years the study of these questions has yielded very suggestive results. The following experiments were undertaken with the idea of enlarging on these so far as the nitrogen metabolism of nephritis is concerned.

METHODS

Dogs were kept in metabolism cages and fed on the required diet. The latter consisted of hashed lean meat, lard, cracker meal, bone-ash and water, given in weighed or measured quantities. Urea and sodium chlorid were added in certain instances. All animals were rejected that did not take kindly to the cage life and food, or that showed any pathologic quantities of albumin in the urine.

The nitrogen was determined by the Kjeldahl process, the sodium chlorid according to Volhard, the total sulphur by Benedict's method,[2] glucose by Benedict's modification[3] of Fehling's method, the non-

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* From the Department of Medicine and the Laboratory of the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York. Under the auspices of the Edward N. Gibbs' Prize Fund of the New York Academy of Medicine.

[1] Von Noorden: Handbuch. der Pathologie des Stoffwechsels, Berlin, 1906, i, 1002.

[2] Benedict: Jour. Biol. Chem., 1909, vi, 363.

[3] Benedict: Jour. Biol. Chem., 1911, ix, 57.

protein nitrogen of the blood according to the procedure of Folin and Denis,[4] and the albumin by the Esbach test.

All the urinary determinations, except those for glucose and albumin, were made on an albumin-free specimen. The urine was acidified with acetic acid, boiled, cooled, made up to double the original volume, filtered and the filtrate used for the analyses. The uranium nitrate was injected subcutaneously in 1 per cent. solution in the amounts noted in the individual protocols. Blood was obtained by aseptic puncture of the heart. Water was given by stomach-tube when necessary. Forty metabolism experiments were performed. Limitation of space forbids the citation of all the protocols.

THE LESIONS OF URANIUM NEPHRITIS

The examination of the kidneys in these animals yielded anatomic pictures which were in full accord with the observations previously published.[5] The tubules showed the most marked changes. The convoluted tubules, especially, showed extensive granular degeneration to be followed by necrosis. Many of the glomeruli were markedly congested, but gave no other evidence of disease. Hence, this form of experimental nephritis has been classified as a tubular nephritis. Though the anatomic study of these kidneys did not demonstrate any notable involvement of the glomeruli, the work of Schlayer and his associates[6] on rabbits, confirmed by Pearce, Hill and Eisenbrey[7] on dogs, proves that there is a functional change in the vascular apparatus, that is, of the glomeruli of the kidney, which may be of far-reaching significance in modifying the activity of that organ. Christian[8] has noted hyaline degeneration of the walls of the capillaries of the glomerular tuft. These changes are so very limited in extent that it does not appear probable that they have an appreciable influence in changing the excretory power of the kidney.

NITROGEN ELIMINATION IN THE URINE—RETENTION

By using large doses of uranium nitrate it was found that the nitrogen in the urine becomes diminished and is consequently retained. This is well shown in Dog 25, Table 1. Such a retention of nitrogen

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[4] Folin and Denis: Jour. Biol. Chem., 1912, xi, 527.

[5] Kraus, Walter M.: The Effect of Uranium Nephritis on the Excretion of Creatinin, Uric Acid and Chlorids, and the Effect of Creatinin Injections during Uranium Nephritis, The Archives Int. Med., 1913, xi, 613, gives a summary of the literature on this subject.

[6] Schlayer and Takayasu: Deutsch. Arch. f. klin. Med., 1910, xcviii, 17. Schlayer, Hedinger and Takayasu: ibid., 1907, xci, 59.

[7] Pearce, Hill and Eisenbrey: Jour. Exper. Med., 1910, xii, 196.

[8] Christian, Henry A., Smith, R. M., and Walker, I. Chandler: Experimental Cardiorenal Disease, The Archives Int. Med., 1911, viii, 468.

TABLE 1.—Result of Experiment with Dog 25 *

Period of Nephritis	Albumin per 1,000 by Esbach Test	Weight kilos	Water Intake c.c.	Volume of Urine c.c.	Urinary Nitrogen gm.	Urinary Sulphur gm.	Ratio N : S	Remarks
Days								
Control...	0.0	8.53	360	450	8.47	0.52	16 : 1	
Control...	0.0	8.58	360	485	8.47	0.45	19 : 1	
Control...	0.0	8.54	360	510	8.01	0.50	16 : 1	
Control...	0.0	8.55	360	445	8.22	0.50	16 : 1	0.10 gm. uranium nitrate injected subcutaneously.
Test								
1	1.5	8.48	360	530	6.21	0.52	12 : 1	
2	...	8.08	360	730	....	....	.....	Vomited.
3	2.2	8.11	560	685	6.31	0.42	15 : 1	
4	2.0	8.19	660	635	6.21	0.44	14 : 1	
5	...	7.58	360	910	....	....	.....	Vomited.
6	1.8	7.48	660	500	4.32	0.30	14 : 1	Vomited.

* The daily diet of this animal consisted of hashed ox-heart, 225 gm.; cracker dust, 60 gm.; lard, 10 gm.; bone ash, 10 gm.; water as noted under "water" in table.

TABLE 2.—RESULT OF EXPERIMENT WITH DOG 23 *

Period of Nephritis	Albumin per 1,000 by Esbach Test	Volume Urine c.c.	Glucose		Urinary Nitrogen gm.	Urinary Sodium Chlorid gm.	Urinary Sulphur gm.	Ratio N : S	Remarks
			Pct.	Gm.					
Days									
Control...	0.0	282	0.0	0.0	2.82	1.40	0.14	20 : 1	
Control...	0.0	318	0.0	0.0	2.69	1.60	0.14	20 : 1	
Control...	0.0	330	0.0	0.0	2.88	1.46	0.14	21 : 1	
Control...	0.0	266	0.0	0.0	3.10	1.11	0.13	23 : 1	
Control...	0.0	320	0.0	0.0	3.06	1.50	0.13	23 : 1	
Test									
1	Trace	310	...	...	3.34	1.32	0.16	21 : 1	0.004 gm. uranium nitrate injected subcutaneously.
2	1.0	360	0.6	2.2	3.20	0.89	0.21	16 : 1	0.004 gm. uranium nitrate injected subcutaneously.
3	3.0	565	1.6	9.0	3.58	0.50	0.28	13 : 1	0.004 gm. uranium nitrate injected subcutaneously.
4	3.0	550	2.8	15.4	4.34	0.58	0.27	16 : 1	0.004 gm. uranium nitrate injected subcutaneously. Vomited a little food.
5	2.0	595	2.3	13.6	4.36	0.82	0.26	17 : 1	0.004 gm. uranium nitrate injected subcutaneously. Vomited a little froth.

* The daily diet of this animal consisted of chopped beef-heart, 120 gm.; cracker dust, 32 gm.; lard, 24 gm.; bone ash, 8 gm.; sodium chlorid, 1 gm.; water, 290 c.c. This diet was fed for nine days before the collection of urine was begun.

TABLE 3.—RESULTS OF EXPERIMENT WITH DOG 10*

Period of Nephritis	Albumin per 1,000 by Esbach Test	Volume Urine c.c.	Glucose		Urinary Nitrogen gm.	Nitrogen Balance †	Urinary Sulphur gm.	Ratio N : S	Remarks
			Pct.	Gm.					
Days §									
Control...	0.0	300	0.0	0.0	5.13	—0.33	0.33	16 : 1	
Control...	0.0	250	0.0	0.0	5.13	—0.33	0.33	16 : 1	
Control...	0.0	250	0.0	0.0	4.52	+0.28	0.29	16 : 1	
Control...	0.0	228	0.0	0.0	4.52	+0.28	0.29	16 : 1	
Control...	0.0	240	0.0	0.0	4.78	—0.02	0.30	16 : 1	
Control...	0.0	280	0.0	0.0	4.78	—0.02	0.30	16 : 1	0.01 gm. uranium nitrate injected subcutaneously.
Test									
1	0.5	290	0.0	0.0	4.48	+0.32	0.29	15 : 1	
2	2.0	258	0.2	0.5	4.73	+0.07	0.32	15 : 1	
3	2.8	393	0.8	3.0	4.69	+0.11	0.34	14 : 1	
4	3.0	410	0.9	3.7	4.28	+0.52	0.31	14 : 1	
5	2.0	342	1.3	4.3	4.42	+0.38	0.31	14 : 1	
6	1.8	302	1.5	4.6	5.01	—0.21	0.31	16 : 1	
7	1.5	290	1.3	3.8	5.60	—0.80	0.30	19 : 1	
8	1.8	310	1.8	5.5	5.98	—1.18	0.29	20 : 1	
9	1.3	385	1.6	6.0	6.96	—2.16	0.33	21 : 1	
10	1.8	350	0.5	1.7	5.83	—1.03	0.27	22 : 1	
11	1.3	345	0.7	2.3	5.88	—1.08	0.26	22 : 1	
12	0.8	314	0.4	1.3	5.47	—0.67	0.26	21 : 1	
13	0.5	280	0.2	0.5	4.64	+0.16	0.23	20 : 1	
14	0.3	260	0.0	0.0	4.81	—0.01	0.24	20 : 1	
15	0.5	324	†	†	5.69	—1.89	0.33	17 : 1	
16	0.3	248	0.0	0.0	4.13	+0.67	0.22	18 : 1	
17	0.3	264	0.0	0.0	4.68	+0.12	0.26	18 : 1	
18	Trace	330	0.0	0.0	5.63	—0.83	0.32	18 : 1	
19	0.0	274	0.0	0.0	4.99	—0.19	0.28	18 : 1	
20	0.0	324	0.0	0.0	5.44	—0.65	0.29	19 : 1	

* The daily diet of this animal consisted of hashed lean beef, 120 gm.; cracker dust, 32 gm.; lard, 24 gm.; bone ash, 8 gm.; water, 280 c.c. This diet, containing 5.30 gm. of nitrogen, was fed for twelve days before specimens of urine were collected for analysis.

† Trace.

‡ 0.5 gm. nitrogen were calculated as being excreted in the feces.

§ During the control days analyses were made every forty-eight hours; subsequently every twenty-four hours.

TABLE 4.—Result of Experiment with Dog 15 *

Period of Nephritis	Albumin per 1,000 by Esbach Test	Weight kilos	Volume of Urine c.c.	Glucose		Urinary Nitrogen gm.	Nitrogen Balance † gm.	Remarks
				Pct.	Gm.			
Days								
Control...	0.0	15.40	860	0.0	0.0	23.43	—0.95	
Control...	0.0	15.40	675	0.0	0.0	20.02	+2.46	
Control...	0.0	15.55	635	0.0	0.0	19.37	+3.11	
Control...	0.0	15.40	870	0.0	0.0	29.28	—6.80	
Control...	0.0	15.54	650	0.0	0.0	20.22	+2.26	0.15 gm. uranium nitrate injected subcutaneously.
Test								
1	0.0	15.43	840	0.0	0.0	23.71	—1.23	
2	1.5	15.36	870	‡	‡	21.00	+1.48	
3	2.8	15.20	870	...	...	23.38	—0.90	Vomited; urine clear.
4	2.0	14.94	974	2.0	19.5	21.47	+1.01	
5	3.0	14.84	838	2.2	18.4	.....	....	Vomited; urine mixed with vomit.
6	1.8	14.54	1,090	2.8	30.5	29.55	—7.07	Vomited; urine clear.
7	1.0	14.46	875	2.3	20.1	24.05	—1.57	
8	1.0	13.79	950	2.8	11.9	24.24	—1.76	Vomited; urine clear.
9	2.0	13.74	890	3.1	27.6	28.14	—5.66	
10	2.0	13.87	815	2.2	17.9	25.27	—2.79	
11	2.5	14.03	595	2.8	16.7	19.94	+2.54	
12	1.8	14.13	685	1.9	13.0	25.06	—2.58	
13	1.0	14.40	640	1.3	8.3	23.71	—1.23	
14	0.5	14.59	605	0.4	2.4	23.09	—0.61	
15	Trace	14.88	535	0.0	0.0	20.51	+1.97	
16	0.0	15.06	645	0.0	0.0	21.50	+0.98	
17	0.0	15.12	730	0.0	0.0	20.54	+1.94	
18	0.0	15.26	650	0.0	0.0	18.04	+4.44	
19	0.0	15.29	765	0.0	0.0	19.74	+2.74	

* The daily diet of this animal consisted of chopped lean beef, 286 gm.; cracker dust, 60 gm.; lard, 30 gm.; bone ash, 15 gm.; sodium chlorid, 2.55 gm.; urea, 11.54 gm.; water, 750 c.c. This diet containing 22.98 gm. of nitrogen was fed for ten days before the collection of urine was begun.

† The amount of nitrogen calculated as being excreted in the feces was 0.5 gm. ‡ Trace.

has been found in various forms of experimental nephritis. Siegel[9] demonstrated this in uranium nephritis, Pearce, Hill and Eisenbrey[7] in experimental tubular nephritis. Austin and Eisenbrey[10] showed that "uranium, cantharidin and potassium chromate in larger doses impair the power of the kidney to eliminate nitrogen." Underhill,[11] and Underhill, Wells and Goldschmidt[12] obtained marked diminution in the excretion of nitrogen in tartrate nephritis, which proved to be a typical tubular nephritis, and Green[13] similar results in chromate nephritis.

There seems to be no doubt that kidney insufficiency in these cases is responsible for the retention of nitrogen. In this animal (Table 1), as throughout these experiments, the quantity of urine excreted was large in amount, and the retention of any urinary constituent cannot be attributed to an oliguria or anuria. In this respect the dog differs widely from the rabbit, which has been a favorite subject for similar work.

NITROGEN ELIMINATION IN THE URINE — INCREASED EXCRETION

With smaller doses of uranium nitrate, yet sufficiently large to produce a distinct nephritis, as evidenced by the degree of albuminuria, the excretion of nitrogen in the urine becomes increased instead of diminished. This may be readily seen in Table 2, Dog 23; Table 3, Dog 10; Table 5, Dog 24; Table 6, Dog 31; Table 7, Dog 33; Table 9, Dog 34; Table 10, Dog 5; Table 11, Dog 27, and Table 14, Dog 1. The amounts of nitrogen excreted in these cases are considerable. Thus Dog 23, a small animal weighing 7.5 kilos, showed, on comparing the urine of the control with the experimental period, an average daily loss of 0.85 gm. of nitrogen during five days of uranium nephritis, that is, an increased excretion of 29.2 per cent. over the control days; Dog 10 (the feces being estimated and not determined), a negative nitrogen balance of 8.35 gm. in twenty days; Dog 34 (the nitrogen content of all the excreta being determined), a loss of 8.26 gm. in nine days, none of this loss occurring in the feces in which the nitrogen content was not increased above that of the control period, and only 0.70 gm. in the vomit.

Pohl,[14] working with rabbits, found a similar condition in subacute uranium nephritis. The increased excretion of water, nitrogen and sodium chlorid in his experiments was ascribed to "an irritation of the kidney." Austin and Eisenbrey[10] found that, in dogs, uranium and cantharidin in the smallest doses capable of producing a nephritis, tend to increase the elimination of nitrogen, probably by stimulating tissue catabolism. Beyond stating this as a surmise no proof of this contention is brought out. In the experiments of Austin and Eisenbrey larger doses of these drugs impaired the power of the kidney to eliminate nitrogen. This was not apparent unless the daily nitrogen intake was high, 1 gm. per kilo. An increase in the nitrogen fed does not necessarily produce a retention of nitrogen. Thus Table 4, Dog 15, gives the protocol of an animal weighing 15.5 kilos receiving 22.98 gm. of nitrogen (11.54 gm. as urea, the remainder in meat and cracker meal) and eliminating this amount without any difficulty; Table 14, Dog 1, shows how approximately 1 gm. of nitrogen per kilo is excreted without retention. In these experiments the severity of the nephritis appeared to be of far greater moment in producing retention of nitrogen than did the quantity of nitrogen fed. It is certain that a nephritis of considerable intensity may be produced without any manifestation of kidney insufficiency so far as the urinary nitrogen is concerned. Individual animals seem to vary greatly as to their susceptibility to the poison and it seems much more rational to measure the severity of the nephritis by the intensity of the symptoms, vomiting, etc., rather than by the dose of uranium given.

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[9] Siegel: Ztschr. f. exper. Path. u. Therap., 1907, iv, 561.

[10] Austin and Eisenbrey: Jour. Exper. Med., 1911, xiv, 366.

[11] Underhill: Jour. Biol. Chem., 1912, xii, 115.

[12] Underhill, Wills and Goldschmidt: Jour. Exper. Med., 1913, xviii, 347.

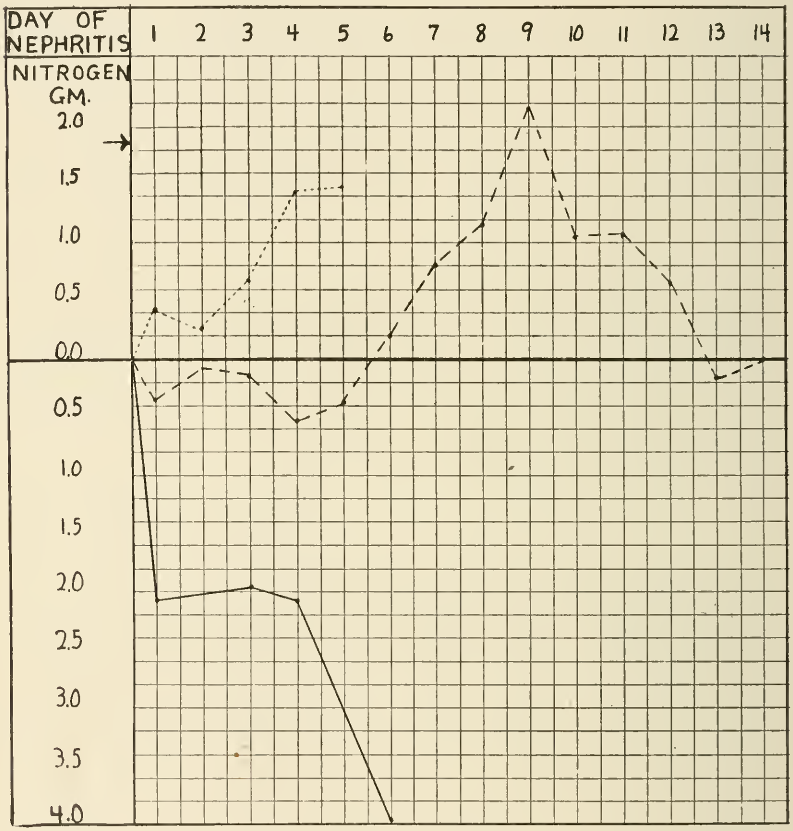
[13] Green: Jour. Path. and Bacteriol., 1909, xiii, 296.

[14] Pohl: Arch. f. exper. Path. u. Pharmakol., 1892, xxix, 282.

The accompanying chart expresses better than words the extreme variation of nitrogen excretion. The curves shown are all typical. Dog 23 showed an increased nitrogen elimination from the beginning of the nephritic period, Dog 25 a retention and Dog 10 a normal or slightly subnormal amount for the first few days, followed by a considerable increase. The last type of elimination is the one which was produced most frequently (many of the protocols are not given), the aim in most of these experiments being to work with an increased elimination of the nitrogen in the urine and to discover its significance.

NITROGEN ELIMINATION IN THE FECES

The quantity of nitrogen found in the feces did not vary from the normal, provided that there was no diarrhea. This is seen in Table 8, Dog 35 (only the first four days of the nephritic period should be taken into account; during the following days the animal refused food and the fecal nitrogen naturally diminished); Table 9, Dog 34, and Table 10, Dogs 5 and 7. These findings are in accord with those of Siegel[9] and Pearce, Hill and Eisenbrey.[7] It is, therefore, permissible, if the feces are of normal consistency, to estimate the nitrogen of the feces as has been done in calculating some of the nitrogen balances. When diarrhea does occur the amounts of nitrogen excreted through the intestines may be enormous. Table 7, Dog 33, shows an excretion in the feces of 7.62 gm. of nitrogen during one day of diarrhea. This amount exceeded the quantity of nitrogen in the food by a small margin. It is worthy of note that the quantities of nitrogen in the feces and in the urine do not bear an inverse ratio to each other, but appear to run an independent course.



Comparative curves of excretion of urinary nitrogen as compared with amount of nitrogen of the control periods. The types of elimination shown. Arrow indicates time of uranium injection. Solid line across chart indicates level of urinary nitrogen during control period; curves above line, excess of excretion; below, retention. Line of dashes, Dog 10; dotted line, Dog 23; solid line, Dog 25.

THE CAUSE OF INCREASED NITROGEN EXCRETION

Two possibilities present themselves: (1) changes in the functional activity of the kidney, (2) increased protein catabolism. The old idea that in every case of nephritis an increased elimination of one of the urinary constituents is preceded by a retention, obviously cannot apply here.

1. *Changes in the Functional Activity of the Kidney.*—It is tempting to associate a definite renal lesion with a correspondingly clear-cut diminution in the excretion of one or more urinary constituents. When these vary so that they may be either increased or diminished, from the very outset of a nephritis, it becomes clear that such an idea must necessarily be revised. Schlayer[15] and his pupils have brought forward some most suggestive theories based on experimental evidence, as well as clinical observations to elucidate this point. They claim that a moderately severe lesion of the kidney parenchyma results in "hypersensitiveness," characterized by an overactivity of the part. Under these circumstances the kidney acts like a suction-cup in hastening the formation of urine. As the pathologic involvement becomes more complete the hypersensitiveness and overactivity diminish in direct ratio to its intensity, until function ceases entirely. If recovery takes place, the stage of hypersensitiveness is again passed through before the kidney returns to normal in its function. That water may be excreted in this manner was proved to the satisfaction of these investigators; sodium chlorid, though not so thoroughly investigated, appeared to follow the same course. Pohl's[14] previous efforts resulted in somewhat similar conclusions regarding the excretion of salt and nitrogen.

These theories seem to indicate that the kidney plays an active part in the excretion of excess water, salt, nitrogen or other substances. Another conception that might explain the phenomenon under consideration is that the augmented production of urinary constituents may be passive rather than active. Barcroft and Straub[16] showed how diuresis could, under certain physiologic conditions, occur without the consumption of oxygen, that is, an increased production of urine occurred with a passive kidney, presumably by a process of filtration. The constant presence of glucose in the urine in experimental uranium nephritis (Table 2, Dog 23; Table 3, Dog 10; Table 4, Dog 15; Table 11, Dog 27, and Table 14; Dog 1), which is due to a renal glycosuria,[17] points toward the possibility that such a kidney may be superpermeable to other substances besides sugar.

These two factors, "hypersensitiveness" and increased permeability may play a secondary rôle in producing the excess of urinary nitrogen found in these experimental animals and possibly in human beings. The primary cause is, in all probability, to be sought in an increased protein catabolism, an excess of waste nitrogen in the blood being presented to the kidney for excretion.

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15. Schlayer: Beiheft. z. med. Klin., Part 9, 1912, viii, 211, gives a summary of these views.

16. Barcroft and Straub: Jour. Physiol., 1910, xli, 145.

17. Frank: Arch. f. exper. u. Pharmakol., 1913, lxxii, 387.

2. *Increased Protein Catabolism.*—In order to determine whether increased protein catabolism was a cause for the negative balances in experimental uranium nephritis, recourse was had to two procedures: (1) determining the sulphur in the urine; (2) estimating the non-protein nitrogen of the blood.

TABLE 5.—Nitrogen, Sulphur and Ratio of Nitrogen to—

Dog No.	23			25		
Diet	Constant containing meat, lard, cracker dust, bone ash and water.			Constant amounts of meat, lard, cracker dust and bone ash. Amounts of water varied.		
Urinary Nitrogen and Sulphur, gm.	N	S	N : S	N	S	N : S
Average of control days.....	2.91	0.14	21 : 1	8.54	0.49	17 : 1
Days of Test						
First	3.34	0.16	21 : 1	6.21	0.52	12 : 1
Second	3.20	0.21 †	16 : 1	....	....	....
Third	3.58	0.28 †	13 : 1	6.31	0.42	15 : 1
Fourth	4.34	0.27 †	16 : 1	6.21	0.44	14 : 1
Fifth	4.36	0.26 †	17 : 1	....	....	....
Sixth	......	......	......	4.32	0.30	14 : 1
Seventh	......	......	......	......	......	......
Eighth	......	......	......	......	......	......
Ninth	......	......	......	......	......	......
Tenth	......	......	......	......	......	......
Eleventh	......	......	......	......	......	......
Twelfth	......	......	......	......	......	......
Thirteenth	......	......	......	......	......	......
Fourteenth	......	......	......	......	......	......
Fifteenth	......	......	......	......	......	......
Sixteenth	......	......	......	......	......	......
Seventeenth	......	......	......	......	......	......
Eighteenth	......	......	......	......	......	......
Nineteenth	......	......	......	......	......	......
Twentieth	......	......	......	......	......	......
Approximate results of observations on: N		+			—	
N : S		—			—	
S		+			=	

† This indicates that the injection of uranium nitrate was repeated.

1. The N : S Ratio: As the result of protein catabolism in the organism there should be an increase in urinary sulphur as well as nitrogen. The N : S ratio should theoretically remain constant. Table 5

summarizes the studies made in this connection. They give no clear-cut results. There are certain evident objections to drawing conclusions concerning the constancy of protein catabolism from the N : S ratio. In the first place a nephritic kidney may be able to eliminate nitrogen readily and retain sulphur, or vice versa (as in Dog 25). In the second place, the various forms of protein destroyed probably contain different percentages in composition of nitrogen and sulphur, and thus do not present a constant ratio. This is especially true when

—Sulphur in the Urine of Dogs in Experimental Uranium Nephritis

10			22			24			27		
Constant containing meat, lard, cracker dust, bone ash and water.			Constant containing meat, lard, cracker dust, bone ash and water.			Constant amounts of meat, lard, cracker dust and bone ash. Amounts of water varied.			Salt solution only. Given by stomach-tube.		
N	S	N : S	N	S	N : S	N	S	N : S	N	S	N : S
4.81	0.31	16 : 1	5.76	0.36	16 : 1	5.75	0.37	16 : 1	2.01	0.14	14 : 1
4.48	0.29	15 : 1	5.43	0.34†	16 : 1	5.76	0.40	15 : 1	2.72	0.24	12 : 1
4.73	0.32	15 : 1	5.38	0.34	16 : 1	4.99	0.38	13 : 1	2.68	0.19	14 : 1
4.69	0.34	14 : 1	4.80	0.33	15 : 1	5.69	0.39†	15 : 1	2.33	0.16	14 : 1
4.28	0.31	14 : 1	4.83	0.40	12 : 1	5.98	0.38	16 : 1	1.65	0.11	15 : 1
4.42	0.31	14 : 1	5.10	0.33	15 : 1	6.51	0.39†	17 : 1	2.30	0.14	17 : 1
5.01	0.31	16 : 1	5.88	0.30	20 : 1	6.12	0.39	16 : 1	2.88	0.18	17 : 1
5.60	0.30	19 : 1	5.88	0.31	20 : 1	7.39	0.45†	16 : 1	2.82	0.19	15 : 1
5.98	0.29	20 : 1	....	....	.....	....	....	.....	3.05	0.18	17 : 1
6.96	0.33	21 : 1	....	....	.....	8.60	0.50	17 : 1	3.61	0.22	16 : 1
5.83	0.27	22 : 1	6.98	0.34	21 : 1	9.42	0.52	18 : 1			
5.88	0.26	22 : 1	5.52	0.34	17 : 1						
5.47	0.26	21 : 1	6.91	0.35	20 : 1						
4.64	0.23	20 : 1									
4.81	0.24	20 : 1									
5.69	0.33	17 : 1									
4.13	0.22	18 : 1									
4.68	0.26	18 : 1									
5.63	0.32	18 : 1									
4.99	0.28	18 : 1									
5.45	0.29	19 : 1									
	+			+			+			+	
	+			+			=			=	
	—			=			+			+	

‡ In this table + means increased; —, diminished; =, constant.

TABLE 6.—Result of Experiment with Dog 31 *

Period of Nephritis	Albumin per 1,000 by Esbach Test	Weight kilos	Volume of Urine c.c.	Urinary Nitrogen gm.	Water Balance c.c.	Nitrogen Balance gm. †	Non-Protein Nitrogen of Blood, mg. per 100 c.c.	Remarks
Days								
Control...	0.0	8.67	340	....	— 60	....	21.6	
Control...	0.0	8.62	338	....	— 58	....		
Control...	0.0	8.62	327	4.29	— 47	+ 1.24	21.8	
Control...	0.0	8.56	370	5.39	— 90	+ 0.14		
Control...	0.0	8.56	345	5.43	— 65	+ 0.10	24.0	0.01 gm. uranium nitrate injected subcutaneously.
Test								
1	1.0	8.44	415	4.84	—135	+ 0.69		
2	2.0	8.17	585	5.35	—305	+ 0.18	35.6	
3	2.0	7.86	630	6.82	—350	— 1.19	51.2	
4	1.7	7.47	640	6.21	—360	— 0.79	68.8	Vomited solid matter containing 0.11 gm. nitrogen.
5	1.5	....	174	1.28	.....	— 1.88	100.0	Ate only part of food. Food eaten contained 3.21 gm. of nitrogen. Vomitus contained 3.21 gm. of nitrogen.

* The daily diet of this animal consisted of chopped beef-heart, 160 gm.; cracker dust, 32 gm.; lard, 16 gm.; bone ash, 8 gm.; water, 280 c.c. This diet containing 6.13 gm. of nitrogen was fed for twelve days before specimens were collected for analysis. The dog was catheterized at the completion of each twenty-four-hour period.

† The amount of nitrogen estimated as being excreted in the feces daily was 0.6 gm.

the ratios of the control periods represent the excretory products of food proteins that have a widely different source from the dog's own tissues. In this connection the results in Dog 27 are significant, as this animal was given only salt and water and no protein food. In this experiment the N : S ratio remained constant, thus pointing toward an increased protein catabolism as the source of the excess of nitrogen eliminated. The results as a whole, however, are inconclusive; like many studies in nephritis they yield figures which are at times constant, at others variable, and it becomes necessary to look further for a more trustworthy evidence on the question in hand.

2. The non-protein nitrogen of the blood: An increase in the non-protein nitrogen of the blood has usually been considered due to retention by a diseased kidney, in both experimental and clinical studies. Thus in the researches of Folin, Karsner, Howard and Denis[18] such an increase found in uranium and cantharidin nephritis has been interpreted as a retention of nitrogen. In the present experiments a similar increase in the non-protein nitrogen of the blood was demonstrated, see Tables 6, 7, 8 and 9. While this increase occurred in the blood, nitrogen was not diminished in the urine, but was excreted in quantities distinctly greater than that taken in in the food. This led to the inevitable conclusion that both the high blood and urinary figures for non-protein nitrogen were due not to kidney insufficiency, but to an increased protein catabolism.

The water metabolism in this connection demands some study. In the protocols of Tables 6 and 7 it is evident that the animals lost a considerable amount of water. This may have resulted in an inspissation of the blood and a consequent rise in the percentage of the non-protein nitrogen of the blood without an absolute increase. The high blood-figures obtained seem to contradict this possibility. Furthermore, as shown in Table 8 and very clearly in Table 9, when extra water was given by stomach-tube, a positive water balance was obtained, and yet the non-protein nitrogen of the blood was markedly higher than in the control periods, thus verifying the conclusions that an increased protein catabolism occurred. In both of these protocols, however, it is perfectly evident that when the water balances are again allowed to become negative the figures for non-protein nitrogen of the blood undergo a corresponding rise. That a water loss or gain to the body is a factor that influences the results in quantitative estimations of the non-protein nitrogen of the blood, becomes apparent.

Another problem presents itself for solution at this point: Why does a kidney retain some of this nitrogen and excrete the

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[18] Folin, Karsner, Howard and Denis: Jour. Exper. Med., 1912, xvi, 789.

TABLE 7.—RESULT OF EXPERIMENT WITH DOG 33 *

Period of Nephritis	Albumin by Esbach per 1,000 Test	Weight kilos	Water Intake c.c.	Volume of Urine c.c.	Urinary Nitrogen gm.	Nitrogen of Vomit gm.	Fecal Nitrogen gm.	Nitrogen Balance gm.	Nonprotein Nitrogen of Blood, mg. per 100 c.c.	Remarks.
Days										
Control...	0.0	....	350	495	....	....	....	.....	28.0	
Control...	0.0	9.93	350	460	4.85	.....	....	.....	....	
Control...	0.0	9.80	350	610	5.97	....	....	.....	....	
Control...	0.0	9.70	350	520	6.65	....	....	.....	22.2	
Control...	0.0	9.60	350	485	6.49	....	....	.....	....	
Control...	0.0	9.68	350	365	6.09	....	....	.....	....	
Control...	0.0	9.63	350	435	6.79	....	....	.....	....	
Control...	0.0	9.60	350	400	6.22	....	....	....	....	
Control...	0.0	9.59	350	395	6.34	....	....	....	....	
Control...	0.0	....	350	400	6.48	....	0.54	—0.98	25.1	
Control...	0.0	9.55	350	395	8.33	....	0.55	—2.84	....	
Control...	0.0	9.48	350	430	7.29	....	0.47	—1.72	....	0.014 gm. uranium nitrate injected subcutaneously.
Test										
1	0.2	9.42	850	930 ‖	7.58	....	....	—1.54	....	
2	1.8	9.17	1,850	1,170 ‖	5.51	1.44	1.31	—2.17	45.6	Diarrhea; vomit.
3	0.4	9.09	1,850	1,350	5.42	....	4.48 ‡	—3.86	56.2	Diarrhea; vomit.
4	0.2	9.08	1,850	1,650	6.94	....	7.62	—8.52	59.4	Diarrhea.
5	0.2	9.03	1,850	1,785	7.36	....	1.10	—2.42	63.2	
6	0.2	8.95	1,850	1,960	7.46	....	....	—1.42	62.5	
7	...	....	350	1,030	8.38	0.06	1.20	—3.60	49.5	Vomit.

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* The daily diet of this animal consisted of chopped beef-heart, 150 gm.; cracker dust, 40 gm.; lard, 20 gm.; bone ash, 10 gm.; the water was varied in amount as indicated under water intake. This diet containing 6.04 gm. of nitrogen was fed for six days before specimens were collected for analysis. The dog was catheterized at the completion of each twenty-four-hour period.

† The water balance could not be calculated because of diarrhea. The figures for weight show that there was a considerable loss of water.

‡ Includes nitrogen of both vomit and feces.

‖ Contaminated with fecal matter.

remainder? Table 4 shows how an animal with a nephritis of considerable intensity can eliminate large amounts of nitrogen without any difficulty. It seems probable, therefore, that the augmented blood nitrogen resulting from protein catabolism, circulates in some abnormal chemical combination which the kidney is unable to excrete. When the disease has been in progress for about four days, this chemical compound may be changed into another, which readily passes through the kidney and causes the large negative nitrogen balances present from about this time on.

To make a practical clinical application of these phenomena, it is evident that in interpreting the significance of an increase of non-protein nitrogen in the blood in nephritis, the following facts must be taken into consideration:

1. Retention of nitrogen by an insufficient kidney.
2. Inspissation of the blood due to loss of water.
3. Increase of protein catabolism.
4. The chemical combination in which the non-protein nitrogen exists in the blood.

The cause for the increased protein catabolism may lie in the fact that uranium may entail pathological changes involving other organs than the kidney. In this respect uranium nephritis in dogs has very suggestive relations to human nephritis. The loss of glucose is too slight to have a far-reaching influence on protein metabolism. Thus the D : N ratio in Table 14, Dog 1, whose diet was carbohydrate-free, is very much below 1, indicating that only a very small fraction of the sugar circulating in the blood is excreted and that thus the loss of sugar with its protein-sparing influence cannot be called to account for all the increased protein catabolism.

NITROGEN EXCRETED IN THE SUCCUS ENTERICUS

With the nitrogen content of the feces unchanged from the normal, that of the urine increased, and the non-protein nitrogen of the blood considerably raised, it is of interest to note what effect these apparently contradictory changes in uranium nephritis may have on certain other phases of the nitrogen metabolism. This was studied for the succus entericus in dogs from which the intestinal secretion was collected by means of a Thiery fistula. For the technic of these operations, the methods of calculation, etc., the reader is referred to a previous paper[19] in which the control periods of these animals were discussed. At that time the following conclusion was reached: nitrogen equivalent to about 35 per cent. of the nitrogen intake, though not necessarily derived directly from it, is daily secreted in the succus entericus of dogs on a mixed diet; about 10 per cent. is excreted in the feces and about 25 per cent. is reabsorbed. It is clear from the protocols given in Table 10 that during the nephritic period the nitrogen content of the succus entericus tended to diminish. What effect such a diversion of nitrogen from its normal path of intermediary metabolism may have on the body's health must at present remain purely conjectural. We are led, however, to speculate on the possibility that the cause of some symptom-complexes, such as uremia, may lie in the many intermediary cycles of metabolism, even when the urinary excretions are seemingly adequate. It is also apparent that the intestines do not supplement the functions of the kidney through vicarious excretion, simply because there is an abnormally large quantity of nitrogen to be eliminated; exactly what factors are involved in producing a diarrhea (see Table 7) and increased fecal nitrogen in certain instances and not in others, is not clear.

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[19] Mosenthal, Herman O.: Jour. Exper. Med., 1911, xiii, 319.

TABLE 8.—Result of —

Period of Nephritis	Albumin per 1,000 by Esbach Test	Weight kilos	Water Intake c.c.	Volume of Urine c.c.	Nitrogen Intake gm.	Urinary Nitrogen gm.
Days						
Control...	...	.....	655	...	9.91	....
Control...	...	17.62	655	735	9.91	....
Control...	0.0	17.60	655	740	9.91	....
Control...	0.0	17.61	655	700	9.91	8.76
Control...	0.0	17.65	655	680	9.91	9.07
Control...	0.0	17.63	655	720	9.91	8.77
Control...	0.0	17.68	655	700	9.91	8.52
Control...	0.0	17.64	655	740	9.91	8.89
Test						
1	0.0	17.57	2,655	2,800	9.91	8.25
2	1.0	17.42	3,055	3,220	9.91	8.59
3	0.5	17.53	3,855	3,710	9.91	9.90
4	Trace	18.14	3,155	2,500	9.91	8.20
5	0.2	18.00	1,500	1,565	‡0.0	4.44
6	0.5	16.83	0	960	‡0.0	3.58
7	0.2	15.98	0	640	‡0.0	4.20
8	0.2	15.76	800	780	‡0.0	5.16
9	Trace	15.70	800	640	‡0.0	3.59

* The daily diet of this animal consisted of chopped beef-heart, 270 gm.; cracker was varied in amount as indicated under water intake. This diet containing 9.91 gm. The dog was catheterized at the completion of each twenty-four-hour period.

|| Ditto marks in this column indicate determinations on composite specimens.

THE PARALLELISM OF NITROGEN WITH OTHER URINARY CONSTITUENTS

It has long been acknowledged that a nephritic kidney will retain certain substances while others may be normally excreted. This is also the case in uranium nephritis. Such a lack of parallelism has been noted in the preceding pages as existing between sulphur and nitrogen (Table 5) and between water and nitrogen (Table 1, Dog. 25).

—Experiment with Dog 35 *

Fecal Nitrogen gm. ‖	Water Balance c.c.	Nitrogen Balance gm.	Nonprotein Nitrogen of Blood, mg. per 100 c.c.	Remarks
....	......	......	31.7	Seventh day after feeding was begun.
....	— 80	......	30.1	Twenty-fourth day after feeding was begun.
....	— 85	......	....	
1.02	— 45	+ 0.13	....	
"	— 25	— 0.18	....	
"	— 65	+ 0.12	....	
"	— 45	+ 0.37	....	
"	— 85	± 0.00	....	0.02 gm. uranium nitrate injected subcutaneously.
0.56	— 145	+ 1.10	....	
1.11	— 145	+ 0.20		
0.91	+ 145	— 0.90	47.8	
1.02	+ 655	+ 0.69	63.8	
0.61	— 65	— 5.05	69.4	
0.86	— 960	— 4.44	85.4	
0.23	— 640	— 4.43	88.9	
0.00	+ 20	—5.16	....	
0.55	+ 160	— 4.13	82.7	

dust, 72 gm.; lard, 54 gm.; bone ash, 18 gm.; sodium chlorid, 2.5 gm. The water of nitrogen was fed for twenty-five days before the collection of urine was begun.
† Trace. ‡ Refused food.

Although several animals were studied very closely in this connection, the protocol of only one is reproduced here (Table 11, Dog 27). All yielded similar results. The nitrogen and sulphur in this case are both excreted in increased amounts during the nephritic period, so that the N : S ratio remains fairly constant (Table 5, Dog 27). While we have an excessive excretion of these two substances, sodium chlorid and the phosphates are retained; the power to excrete the former being recovered distinctly sooner. The same discrepancy between nitrogen and sodium chlorid elimination is seen in Table 2, Dog 23. Another inter-

TABLE 9.—RESULT OF EXPERIMENT WITH DOG 34 *

Period of Nephritis	Albumin per 1,000 by Esbach Test	Weight kilos	Water Intake c.c.	Volume of Urine c.c.	Urinary Nitrogen gm.	Nitrogen of Vomit gm.	Fecal Nitrogen gm.	Water Balance c.c.	Nitrogen Balance gm.	Non-protein Nitrogen of Blood mg. per 100 c.c.
Days										
Control...	0.0	12.25	475	490	....	....	....	......	......	35.3
Control...	0.0	12.28	475	470	....	....	....	......	......	....
Control...	0.0	12.22	475	505	....	....	‡			
Control...	0.0	12.23	475	465	....	....	....	......	......	....
Control...	0.0	12.31	475	480	....	....	....	......	......	33.7
Control...	0.0	12.21	475	560	....	....	....	......	......	....
Control...	0.0	12.26	475	470	6.47	....	0.62	+ 5	—0.12	....
Control...	0.0	12.22	475	485	5.91	....	"	— 10	+0.44	....
Control...	0.0	12.29	475	470	6.36	....	"	+ 5	—0.01	....
Control...	0.0	12.23	475	490	6.50	....	"	— 15	—0.15	....
Test §										
1	0.3	12.13	975	1,050	6.57	....	0.56	— 75	—0.16	....
2	0.4	12.10	1,975	1,970	6.44	....	0.69	+ 5	—0.16	40.9
3	0.2	12.46	2,375	2,020	6.38	....	0.55	+355	+0.04	61.3
4	Trace	12.54	2,375	2,165	6.59	....	"	+210	—0.17	66.5
5	Trace	12.64	2,525	2,430	7.60	....	"	+ 95	—1.18	56.8
6	0.2	12.34	2,525	2,690	8.19	....	0.59	—165	—1.81	41.5
7	0.5	11.48	475	1,090	6.39	....	0.56	—615	+0.02	63.8
8	0.5	11.17	475	830	8.29	....	0.59	—355	—1.91	77.4
9	...	10.83	475	720	8.61	0.70	"	—245	—2.93	75.6

* The daily diet of this animal consisted of chopped beef-heart, 195 gm.; cracker dust, 52 gm.; lard, 24 gm.; bone ash, 13 gm.; sodium chlorid, 2 gm.; the water was varied in amount as indicated under water intake. This diet, containing 6.97 gm. of nitrogen, was fed for nine days before the collection of urine was begun. The dog was catheterized at the completion of each twenty-four-hour period.

‡ The ditto marks in this column indicate determinations on composite specimens.

§ At this point 0.015 gm. uranium nitrate was injected subcutaneously.

TABLE 10.—NITROGEN OF THE URINE, FECES AND SUCCUS ENTRICUS OF TWO DAYS WITH EXPERIMENTAL URANIUM NEPHRITIS *

Dog No.	Period of Nephritis	Albumin per 1,000 by Esbach Test	Nitrogen for twenty-four hours, gm.						Remarks
			Intake	Urine ‡	Feces ‡	Succus Entericus	Balance of Nitrogen †		
							Urinary	Succus Entericus	
5	Control...	0.0	7.35	5.81	0.73	2.99			
	Control...	0.0	7.35	"	"				
	Control...	0.0	7.35	"	"	1.59			
	Control...	0.0	7.35	"	"	1.34			
	Control...	0.0	7.35	"	"				
	Days 1	0.8	7.35	5.96	0.61	0.64	— 0.15	+ 1.33	0.01 gm. uranium nitrate injected subcutaneously.
	2	2.0	7.35	5.45	"	0.96	+ 0.36	+ 1.01	
	3	3.0	7.35	9.17	"	0.99	— 3.36	+ 0.98	
	4	2.0	7.35	6.32	"	0.31	— 0.51	+ 1.66	
	5	...	7.35	....	"	0.83	....	+ 1.14	Vomited.
	6	...	0.00	....	0.74	0.28	....	+ 1.69	Fed water only.
	7	0.5	7.35	....	"				
	8	0.5	7.35	....	"	0.90	....	+ 1.07	
	9	0.3	7.35	7.51	"	0.47	— 1.70	+ 1.50	
	10	0.3	0.00	6.65	0.47	....	— 0.84	....	Refused to eat or drink.
7	Control...	0.0	4.33	3.44	0.31	1.80			
	Control...	0.0	4.33	2.93	"	1.23			
	Control...	0.0	4.33	"	"	1.06			
	Control...	0.0	4.33	"	"	0.94			
	Control...	0.0	4.33	"	"	1.00			
	Days 1	0.5	4.33	3.19	0.30	0.86	— 0.16	+ 0.35	0.01 gm. uranium nitrate injected subcutaneously.
	2	4.0	4.33	3.29	"	0.52	— 0.26	+ 0.69	
	3	4.5	4.33	3.30	"	0.47	— 0.27	+ 0.74	
	4	4.5	4.33	3.31	"	0.58	— 0.28	+ 0.63	
	5	5.0	4.33	3.61	"	1.04	— 0.58	+ 0.17	
	6	5.5	4.33	3.85	"	0.84	— 0.82	+ 0.37	
	7	2.0	4.33	2.80	"	1.01	+ 0.23	+ 0.20	
	8	2.5	4.33	....	"	1.63	....	— 0.42	Vomited.
	9	0.8	4.33	4.55	"	2.49	— 1.52	— 1.28	
	10	0.5	4.33	3.45	"	0.98	— 0.42	+ 0.23	
	11	1.5	4.33	3.35	"	0.46	— 0.32	+ 0.75	

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* Both animals were kept on a constant diet of weighed amounts of hashed lean beef, lard, cracker dust and bone ash and measured quantities of water. Dog 5 was kept on such a diet ten days, Dog 7, seven days, before specimens were collected for analysis.

TABLE 11.—RESULT OF EXPERIMENT ON DOG 27 *

Period of Nephritis	Albumin per 1,000 by Esbach Test	Volume Urine c.c.	Glucose		Urinary Nitrogen gm.	Urinary Sulphur gm.	Urinary Sodium Chlorid gm.	Urinary Phosphates gm.	Remarks
			Pct.	Gm.					
Days									
Control...	0.0	325	0.0	0.0	1.91	0.15	1.64	0.53	
Control...	0.0	390	0.0	0.0	1.99	0.14	....	0.55	
Control...	0.0	330	0.0	0.0	2.14	0.14	1.55	0.55	0.02 gm. uranium nitrate injected subcutaneously.
Test									
1	1.0	515	0.7	3.6	2.72	0.24	1.60	0.84	
2	2.0	300	2.0	6.0	2.68	0.19	0.88	0.77	
3	1.0	495	0.7	3.5	2.33	0.16	0.39	0.37	
4	0.5	250	0.6	1.5	1.65	0.11	0.73	0.20	Vomited 95 c.c. of fluid.
5	0.5	290	0.7	2.0	2.30	0.14	0.54	0.24	
6	0.5	320	0.9	2.9	2.88	0.18	0.94	0.31	Vomited 55 c.c. of fluid.
7	0.5	415	0.6	2.5	2.82	0.19	1.21	0.20	
8	0.5	440	0.6	2.6	3.06	0.18	2.53	0.29	
9	0.7	530	0.7	3.7	3.61	0.22	3.24	0.32	

* This animal was given water and 1.6 gm. of sodium chlorid daily. The dog was catheterized at the completion of each twenty-four-hour period.

esting example of this lack of parallelism occurs when, with an excessive total nitrogen excretion, there is a retention of uric acid, one of the constituent nitrogenous substances, as shown in Table 14, Dog 1. Kraus[5] demonstrated an increase of uric acid excretion in dogs with uranium nephritis. In view of the variation found in the elimination of urinary nitrogen it would seem not at all improbable that both conditions, an increased, as well as a diminished uric acid excretion, might be found in cases of uranium nephritis of varying intensity. From the discussion as to the cause of extrarenal nitrogenous metabolism it becomes clear that, while many of the instances of lack of parallelism in the excretion of urinary elements may be ascribed definitely to the local lesions in the kidney, there are others that are dependent on remote metabolism changes.

TABLE 12.—Blood-Pressure Readings in Millimeters of Mercury on Dog 1

	No. of Readings	Maximum	Minimum	Average
Before injection of uranium nitrate, thirteen days	3	90	85	88
During period of albuminuria, following the injection subcutaneously of 0.015 gm. uranium nitrate, seven days	7	130	95	117
Subsequent period, six days	4	100	90	96
During period of albuminuria, following a second subcutaneous injection of 0.015 gm. of uranium nitrate, four days	4	120	105	113
Subsequent period, twenty-nine days	8	110	105	107
During period of albuminuria, following a third subcutaneous injection of uranium nitrate; 0.02 gm. injected, fifteen days	12	140	105	122
Subsequent period, twenty-three days	11	120	100	113
During period of albuminuria, following a fourth subcutaneous injection of uranium nitrate; 0.02 gm. injected, seven days	6	135	120	125

INCREASE OF BLOOD-PRESSURE IN URANIUM NEPHRITIS

The blood-pressure was determined by a modified Riva-Rocci method devised by Janeway.[20] It was extensively studied on two dogs, Dog 1, Table 12; Dog 8, Table 13, and for shorter periods on others.

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[20] Janeway: Proc. Soc. Exper. Biol. and Med., 1909, vi, 108.

Both tables show a rise of blood-pressure which is most marked during the periods of uranium nephritis, but which does not return completely to normal when the albuminuria has ceased. In this way a distinct permanent rise of blood-pressure was obtained in one animal after three nephritic periods, in the other after seven. Concomitant determinations of urinary nitrogen, uric acid and blood-pressure followed for thirty-two days. Table 14, Dog 1 shows that the blood-pressure was maintained at its greatest height while there was an increased protein catabolism as indicated by the increased amounts of nitrogen in the urine, and also a persistent retention of uric acid. It is probable that the increased protein catabolism and the rise of blood-pressure are in some way related, and that the disturbance leaves some permanent impairment of the organism, is indicated by the fact that the blood-pressure remains high even though the nitrogen of the urine returns to its normal level. The kidneys of these two animals did not show the changes of chronic nephritis, on gross inspection or on microscopic examination. Dog 8 showed a very few small areas of round-cell infiltration, such as might very well have been present in any animals that had not been subject to repeated attacks of nephritis; in Dog 1 only the changes of an acute uranium nephritis were found, the animal being killed seven days after the last injection.

TABLE 13.—Blood-Pressure Readings in Millimeters of Mercury on Dog 8

Date 1910	Gm. of Uranium Nitrate Injected Subcutaneously	No. of Readings	Maximum	Minimum	Average
7/24-8/8	(Control Period)	8	100	85	91
7/12	0.01				
10/4	.....	1	90	90	90
10/18	0.01				
10/31	.....	1	105	105	105
11/17	0.015	1	105	105	105
12/2	.....	1	110	110	110
1911					
3/8-3/15	.....	3	120	110	115
3/15	0.015				
4/ 5-4/7	.....	3	120	115	117
7/15-7/17	.....	3	115	110	112
7/19	0.015				
9/19	0.015				
11/3	0.015				
11/3-11/26	.....	4	125	115	119

CONCLUSIONS

In experimental uranium nephritis, nitrogen excretion may be increased or diminished in amount; an increased elimination is due

TABLE 14.—Result of Experiment on Dog 1 *

Period of Nephritis	Albumin per 1,000 by Esbach Test	Weight kilos	Volume of Urine c.c.	Glucose		Urinary Nitrogen gm.	Urinary Uric Acid gm.	Blood-Pressure mm. of Mercury
				Pct.	Gm.			
Average of three control days †	0.0	10.64	365	0.0	0.0	9.24	0.07	108
Test								
1	0.1	10.42	635	0.1	0.6	12.42	0.13	105
2	0.2	10.52	295	0.2	0.6	5.36	0.05	110
3	1.0	10.22	700	1.0	7.0	13.30	0.03	110
4	1.3	10.13	510	1.3	6.6	10.26	0.02	
5	1.9	9.85	685	1.9	13.0	14.74	0.03	125
6	1.5	9.76	535	1.5	8.0	12.11	0.02	135
7	1.3	9.61	520	1.3	6.8	12.20	0.02	140
8	1.0	9.63	480	1.0	4.8	10.06	0.02	125
9	1.2	9.55	620	1.2	7.4	12.30	0.02	125
10	1.8	9.37	590	1.8	10.6	12.61	0.02	120
11	0.8	9.38	420	0.8	3.3	11.04	0.01	120
12	0.5	9.53	305	0.5	1.5	8.44	0.01	125
13	0.3	9.65	315	0.3	0.9	11.03	0.04	120
14	0.1	9.62	410	0.1	0.4	10.14	0.05	
15	0.0	9.74	270	0.0	0.0	6.26	0.06	115
16	0.0	9.75	360	0.0	0.0	7.95	0.06	100
17	0.0	9.77	295	0.0	0.0	‡ 8.99	‡ 0.07	
18	0.0	9.73	390	0.0	0.0	“	“	
19	0.0	9.74	305	0.0	0.0	7.19	0.06	105
20	0.0	9.65	280	0.0	0.0	“	“	
21	0.0	9.80	215	0.0	0.0	8.80	0.07	
22	0.0	9.72	385	0.0	0.0	“	“	105
23	0.0	....	255	0.0	0.0	6.95	0.06	115
24	0.0	9.75	285	0.0	0.0	“	“	115
25	0.0	....	265	0.0	0.0	8.69	0.11	115
26	0.0	....	290	0.0	0.0	“	“	
27	0.0	9.73	205	0.0	0.0	9.80	0.10	120
28	0.0	9.76	300	0.0	0.0	“	“	
29	0.0	....	310	0.0	0.0	8.00	0.08	115
30	0.0	9.78	160	0.0	0.0	“	“	
31	0.0	....	280	0.0	0.0	9.19	0.09	115
32	0.0	9.80	275	0.0	0.0	“	“	

* The daily diet of this animal consisted of hashed lean beef, 250 gm.; lard, 50 gm.; bone ash, 15 gm.; water, 400 c.c. This diet was fed for twenty-one days before specimens were collected for analysis.

† At this point 0.02 gm. uranium was injected subcutaneously.

‡ From this point on the ditto marks indicate that the urine analyses were performed on a specimen collected in an interval of forty-eight hours instead of during twenty-four as previously.

not to any previous retention, but to an augmented protein catabolism, a diminished excretion to kidney insufficiency.

The laws governing the elimination of nitrogen in the urine in nephritis are not haphazard or "bizarre," but such other factors as food intake, normal stools, etc., being constant, depend largely on three conditions: the degree of protein catabolism, the chemical combination in which the non-protein nitrogen exists in the blood and the permeability of the kidney.

The normal nitrogen content of the feces, except during the rare occurrences of diarrhea, the diminished amount of nitrogen in the succus entericus, the increase in the non-protein constituents of the blood, the lack of parallelism between nitrogen and other urinary constituents, and the increased blood-pressure, make it clear that in uranium nephritis we have a disease which implicates the organism as a whole and not the kidneys alone; furthermore, that the various functions of the body do not necessarily supplement each other by vicarious excretion, etc., as is often assumed, but that each one to a great extent follows its own independent laws. Human nephritis presents such a many-sided picture that it seems probable that many of the facts worked out for experimental uranium nephritis are applicable to it. The only claim that can be made for them at present, however, is that they be considered as suggestions on which to base further observations.

In the interpretation of an increased non-protein nitrogen of the blood in nephritis, four factors are to be considered: (1) retention of nitrogen by an insufficient kidney; (2) inspissation of the blood, due to loss of water; (3) increase of protein catabolism; (4) the chemical combination in which non-protein nitrogen exists in the blood. Hitherto general attention has been paid only to the first of these.

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FAMILY PERIODIC PARALYSIS

REPORT OF A TYPICAL CASE, WITH METABOLISM STUDY *

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This is a curious and rare disease of which we have as yet only a few reports in the literature, most of which have been from this country. The first report appears to have been that of Westphal[1] in 1885. Cousot reports five cases, Goldflam[2] nineteen and Taylor eleven cases. Taylor,[3] in his exhaustive paper published in 1898, collected at that time fifty-three cases. Since then a few other cases have been reported, three by J. K. Mitchell, Flexner and Edsall.[4] Last year Gardner[5] reported a single isolated case which he studied in an interesting way. Dr. L. Pierce Clark[6] last year reported a new subtype of this palsy. The most important and extensive observations, however, have been made by Dr. G. E. Holtzapple,[7] a general practitioner of York, Pa., and despite the subsequent reports, Holtzapple's paper still constitutes the most important contribution to the subject. His paper is based on observations running over a period of twenty-two years made on one family in four generations. In this family he has observed seventeen cases of family periodic paralysis, eighteen cases of sick headache and five cases of paralysis and headache. Fourteen members of this family have had paralysis only and thirteen have had attacks of headache only. The total number afflicted with

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* Submitted for publication June 17, 1914.

* From the Wards of the Allegheny General Hospital and the Laboratory of Dr. J. P. McKelvy, Pittsburgh.

[1] Westphal, C.: Ueber einen merkwürdigen Fall von periodischer Lähmung aller vier Extremitäten mit gleichzeitigen Reloschen der elektrischen Erregberkeit während der Lähmung, Berl. klin. Wchnschr., 1885, xxii, 489, 509.

[2] Goldflam, S.: Ueber eine eigenthumliche Form von periodischer, familiarer wahrscheinlich autointoxicatorische Paralyse, Ztschr. f. klin. Med., 1891, xix, Supplement, 240; Weitere Mittheilung über die paroxysmale, familiare Lähmung, Deutsch. Ztschr. f. Nervenh., 1896, vii, 1; ibid., 1897, xi, 242.

[3] Taylor, E. W.: Family Periodic Paralysis, Jour. Nerv. Ment. Dis., 1898, xxv, 637 and 719.

[4] Mitchell, Flexner and Edsall: Brain, 1902, xxv, 109.

[5] Gardner, H. W.: A Case of Periodic Paralysis, Brain, 1913, xxxv, 243.

[6] Clark, L. Pierce: Remarks upon the Irregular and Unusual Types of Familial Periodic Paralysis and Conditions Simulating the Same with a Preliminary Report upon a New Subtype of this Palsy, Review of Neurology and Psychiatry, 1913.

[7] Holtzapple, G. E.: Periodic Paralysis, Jour. Am. Med. Assn., 1905, xlv, 1224.

either paralysis or headache was thirty-two. Of the seventeen members who have suffered from attacks of periodic paralysis, ten were still living at the time of Holtzapple's report. Six of the seven who died succumbed in an attack of paralysis, one of them in Holtzapple's presence. Of this paralytic group, fourteen were under his personal observation. The disease was transmitted through the father in six instances and through the mother in four instances; through the father having had attacks of sick headache only in four instances and through the mother having had attacks of sick headache only in three instances. In nine cases the disease was transmitted through the father, and eight through the mother. Holtzapple describes the attacks as follows:

> This affection in its typical form is characterized by periodic flaccid motor paralysis, involving all of the voluntary muscles, except those of the face, eyes, tongue, organs of speech, of deglutition and the sphincters of rectum and bladder. The attacks may be distinctly periodic for a time and at short intervals, especially in young adults, but frequently later in life at much longer and irregular intervals. The paralysis may be partial or complete, localized or general; the upper extremities alone or only the lower may be involved. It may be confined to the neck, or one-half of the body may be completely paralyzed, while partial paralysis affects the other half. The paralysis may be partial in the morning and become complete during the day.
>
> During an attack of paralysis there may be one or more periods of marked improvement, usually of a few hours' duration, followed by complete helplessness. In a typical attack of complete general paralysis the patient is utterly helpless, cannot move a finger or toe, can neither lift nor turn the head on the pillow, and if unsupported, the head either drops on the sternum or backward between the scapulae. In some instances breathing becomes distinctly labored, and deep breathing, coughing and vomiting impossible. Speech and deglutition are in rare instances affected. The order of involvement is not always the same. It most frequently begins in the muscles of the lower extremities, but may only involve those of the upper or the muscles of the neck. There are no sensory symptoms, except in some instances formication and numbness in the paralyzed parts, also a sense of heaviness and a tired feeling, which at times become very annoying to the patient, necessitating frequent change of posture, to the great relief of the patient.

Holtzapple states that "the duration of an attack may be a few hours, one, two or in rare instances, three full days." Recovery may be rapid, or it may require three or four hours or even half a day. "It is nothing uncommon for one of these patients to be as helpless as a log at 7 a. m. and at 11 a. m. be engaged in doing a hard day's work, not feeling the slightest inconvenience from having been completely paralyzed a few hours before."

The frequency of attacks varies greatly in different members of the family and also in the same individual at different periods. Attacks are most frequent between puberty and the middle period of life; later they become less frequent and more irregular. During an attack the mind remains perfectly clear. Neither cranial nerves nor special senses are involved and the patient retains full control of bladder and rectum.

During severe attacks the pulse is weak and irregular and there is evident cardiac dilatation. During the intervals between attacks the members of the family seem perfectly well.

The hereditary nature of the attacks seems thoroughly established inasmuch as every one of those paralyzed had either a father or mother who was subject to either similar paralytic attacks or sick headache or both. The headache attacks are very similar to those of ordinary hemicrania.

Holtzapple studied 139 specimens of urine, seventy-three specimens from six members of the paralytic group, thirty-six specimens from three individuals who have characteristic headache and thirty specimens from persons who were the picture of health. He found that the members of the paralytic group were not excreting normal quantities of metabolic products. In all of Holtzapple's cases an attack was brought on by overeating.

The case which Gardner reported appears to be a perfectly typical one, although there is no history of heredity in the family. He believes that the disease is due to a congenital defect of metabolism. Dr. Head offered the suggestion to Dr. Gardner that the disease might be an expression of anaphylactic reaction to some form of food such as pork. And although it was known that attacks have occasionally followed the eating of pork sausage, Gardner did not feel justified in making this experiment in his case, as, if the theory were true, the experiment would not be without danger.

Gardner summed up his reasons for believing the attacks due to presence of toxins because of some defect in metabolism as follows:

1. The many points of resemblance to other conditions which are undoubtedly due to auto-intoxication.

2. The sudden onset and the rapid recovery.

3. The invariable occurrence of the phenomena after sleep, when waste and toxic products may be assumed to accumulate, just at the time that intestinal digestion is taking place.

4. The fact that the condition might occasionally be walked off.

5. The symmetrical distribution of the paralysis.

6. The occurrence of headache, drowsiness, thirst, anorexia, aching of the limbs and sweating during the attacks. These toxic symptoms closely resemble those which obtain in other transitory toxemias.

7. The fact that previous attacks of headache, in all probability toxemic, had been replaced by the attacks of paralysis.

8. The high specific gravity of the urine passed during the attacks, and the presence in it of small quantities of indican.

9. The presence of high pulse tension with accentuated aortic second sound and slow pulse in the initial stages and the cardiac dilatation which so rapidly followed.

And acting on this theory he prescribed for his patient as follows:

1. The patient was to give up drinking beer altogether, and to avoid all rich food; and he was to drink large quantities of water.

2. To take magnesium sulphate, 1 dram, in half a pint of hot water on rising every morning.

3. To take compound pill of mercurous chlorid, 2 grains, once a fortnight at bedtime, fellowed by Epsom salts in the morning.

4. To take tincture of digitalis, 4 minims; potassium acetate, 20 grains; syrup, ½ dram, and peppermint water, 1 dram, three times a day.

5. To take caffein citrate, 7½ grains; potassium bromid, 10 grains; diluted hydrobromic acid, 15 minims; syrup 1½ dram, and chloroform water, ½ dram, in a tumbler of water whenever any warning symptoms of an attack made their appearance.

He writes that the attacks which had been getting more and more frequent and threatening to incapacitate the patient at once ceased after the institution of this treatment.

The record of our own case may be inserted at this point:

History.—The patient is a healthy-looking man aged 21, single, habits good. He does not drink or use tobacco. He denies specific disease of any kind. Occupation, foreman in rubber works; he formerly worked at blacksmith's trade. He works ten hours a day. He drinks a glass of wine occasionally.

Patient's great grandmother and grandfather on his mother's side suffered from periodic paralysis, the latter dying of it. A second cousin on his mother's side (a man in middle life) suffered with this disease for many years and suffers from it now. In this cousin the disease was never as severe as in the patient and of late years has grown a good deal less severe.

The patient for the last seven years has been subject to periodic attacks of paralysis. These attacks are of irregular occurrence lasting from a half hour up to three or four days. Of the severe and more prolonged attacks he has had about three or four in a year. He experiences some kind of an attack two or three times every week. They always come on in the night. He wakens in the morning to find himself paralyzed in some part of his body; or he may waken in the night to find himself paralyzed. In severe attacks he is paralyzed in all parts of the body below the head (including the neck). The muscles of the face, tongue, eyes and throat have never been affected. He may recover within half an hour or an hour completely, even from a severe attack. During a severe attack he has only slight motion in the various members of his body. In lighter attacks he wakens in the morning to find some part of his body paralyzed. It may be one leg, or both legs, an arm or both arms, or an arm and leg on the same side or an arm on one side and a leg on the other. Neck muscles alone may be paralyzed. The legs are rather more commonly paralyzed than other parts of the body. In lesser attacks, when he wakens paralyzed, he goes about his work and gradually recovers — so gradually that he is not able to state just when improvement began; and usually about 8 or 9 o'clock in the morning he has entirely recovered. Recalling some of the more severe attacks, he states that on the evenings preceding them he has always had a hearty meal and he attributes these attacks to the meals. He has never had pain or sensory symptoms of any kind. He has never seemed feverish or delirious, or in any way mentally upset.

The patient was admitted to the Allegheny General Hospital September 30, paralyzed all over his body, with only feeble movements in arms and legs. He was unable to raise his arms above his head, to stand or even turn in bed. This attack, which was a major one, had begun about sixty hours previously.

Examination.—Oct. 1, 1914, 2 p. m.: There is almost complete paralysis of the left arm. The right arm is paralyzed to a little less degree. There is but slight movement in legs. The patient is able to flex the feet, but not to extend them. The neck muscles are very weak. He is unable to raise the head from the bed and has only feeble power in throwing the head backward. The muscles of respiration are weak. The diaphragm is weak and he can protrude the abdomen only feebly. The knee-jerks are much reduced — quite feeble. Sensation as to temperature, pain and contact seem everywhere normal. The movements of the head and face all seem normal, also those of tongue and eyes. There is no defect of articulation, or in swallowing. There is no muscular atrophy. The patient's mind seems perfectly clear. The pulse is about 75; the blood-pressure seems somewhat reduced.

October 2, 2 p. m.: The nurse records that at 12 o'clock last night the patient seemed even weaker than in the afternoon and unable to turn himself in bed. Pulse about 50. When she observed him at 2 in the morning he seemed to have fully regained his strength.

The patient seems possessed of his normal strength of all parts of his body. He gives a firm grasp of the hand, and the legs are quite strong. There is no wasting of any of the muscles. The knee-jerks, which yesterday were very much reduced, are quite active to-day, forming a marked contrast to those of yesterday. Dr. Rosenbloom saw him, gave directions for food and diet and for examination of urine and feces. An attempted lumbar puncture was unsuccessful.

Observation at the Hospital.—Shortly after the patient was put to bed his pulse went down to 50 and continued low during the week he was in the hospital, ranging from 50 to 60. A day or two before leaving I joked with him, saying, "Why don't you get up an attack of paralysis, which you say you can do so readily?" His reply was, "You just give me a good, hearty meal to-night and I will guarantee to be paralyzed to-morrow morning."

It would seem that we need look for no further explanation of his freedom from attacks in the hospital than the diet which he took while there.

During his stay of one week in the hospital the patient seemed to be, after the first day, normal in every way. The blood-pressure was:

October 3: Systolic, 68; diastolic, 60.
October 4: Systolic, 68; diastolic, 55.
October 5: Systolic, 90; diastolic, 65.
October 7: Systolic, 92; diastolic, 72.
October 9: Systolic, 95; diastolic, 75.

From consideration of the review of the literature it will be noted that our own case is a highly typical one, and conforms in the main to the criteria laid down by Holtzapple. The attacks seem to be perfectly typical and much more frequent than in most of the cases reported; heredity is plain and clear. It is also like other cases in other respects as follows: The attacks always come on at night; they are often, if not always, preceded by a heavy meal; paralysis comes on during sleep; paralysis wears off by exercise; attacks last from a few hours to and never exceeding three days; there is absence of mental symptoms; there is absence of sensory symptoms; the paralysis is of flaccid character with absence of knee-jerks; the health of the patient between attacks is perfect. Our case also lends strong support to the views expressed by Gardner regarding the nature of the disease, namely, that it is probably due to some congenital defect of metabolism.

Supporting this view are the facts that undoubtedly attacks have been produced (if not always) by hearty meals; and supported further by the fact that while in the hospital and on a limited diet the patient was free from attacks.

TABLE 1.—The Nitrogen Metabolism—

Day	Urine †								
	Volume c.c.	Nitrogen gm.	Urea-Nitrogen		Ammonia-Nitrogen		Creatinin ‡		
			Gm.	Per Cent. of Total Nitrogen	Gm.	Per Cent. of Total Nitrogen	Gm.	Nitrogen	Percentage of Total Nitrogen
1	1,300	14.9	12.6	84.6	0.51	3.4	0.75	0.275	1.8
2	2,125	15.2	12.9	84.5	0.55	3.6	0.86	0.314	2.06
3	1,200	15.4	13.1	85.1	0.62	4.0	0.97	0.354	2.3
4	1,550	15.5	13.0	83.9	0.64	4.1	0.70	0.256	1.7
5	1,420	15.8	13.3	84.1	0.75	4.8	0.86	0.314	2.0
6	1,400	15.1	12.9	85.4	0.66	4.6	0.67	0.244	1.6
7	1,240	15.2	13.3	87.5	0.72	4.7	0.78	0.285	1.9

* Creatin was absent from all specimen of urine.

† At no time did the urine contain acetone, diacetic acid, beta-oxybutyric acid, excess of

‡ Average creatinin coefficient = 4.8 mg. creatinin-nitrogen per kilogram of body weight.

ABSTRACT OF METABOLISM STUDY BY DR. ROSENBLOOM

We have studied in this patient, in a seven-day metabolism experiment, the nitrogen metabolism and urinary nitrogen partition, the sulphur metabolism and urinary sulphur partition, the calcium, magnesium, phosphorus and fat metabolism. We have found a very low creatinin excretion and a very high "undetermined nitrogen" excretion; these findings may indicate the type of metabolic perversion which accounts for the paralytic seizure.

This condition has so many characteristics of a toxemia that it was thought that a complete metabolic study of this patient might throw some light on the disease. Some work has been carried out in the field already. Holtzapple found a marked diminution in the average quantity of urea eliminated by his paralytic group. As no attention was paid to diet and as the hypobromite method for the urea estimations was used, little reliance can be placed on this observation. Goldflam found that during the attacks the toxicity of the urine was increased as determined by Bouchard's method. This method has so many pitfalls that this result is of doubtful value. Singer and Goodbody[8] estimated the amounts of nitrogen, urea, uric acid, chlorids, total sulphates, alkaline sulphates, ethereal sulphates and phosphates, in the urine of the case studied and found no marked changes in the amounts of these substances excreted. They did find a lessened amount of urine excreted during the attacks.

—AND URINARY NITROGEN PARTITION *

Urine †					Feces		Intake	Balance
Uric Acid			Undetermined Nitrogen		Nitrogen		Nitrogen	Nitrogen
Gm.	Nitrogen	Percentage of Total Nitrogen	Gm.	Pct. of Total Nitrogen	Gm.	Pct. of Nitrogen Intake	gm.	gm.
0.42	0.14	0.94	1.37	9.2	1.90	12.1	15.6	— 1.2
0.30	0.10	0.66	1.34	8.8	1.90	10.1	18.8	+ 1.7
0.36	0.12	0.78	1.21	7.9	1.90	11.8	16.2	— 1.1
0.39	0.13	0.84	1.47	9.5	1.90	11.6	16.4	— 1.0
0.36	0.12	0.76	1.32	8.4	1.57	9.4	16.7	— 0.67
0.33	0.13	0.86	1.17	7.7	1.57	9.3	16.9	+ 0.23
0.33	0.13	0.85	0.76	5.0	1.57	9.6	16.4	— 0.37

indican or any other abnormal constituent.

Crafts and Irwin[9] asserted that they had extracted from feces passed immediately after an attack, a substance which produced temporary paralysis when injected into guinea-pigs and rabbits. They also found that the amount of urea excreted during an attack appeared to be diminished. They also estimated in the urine the amounts of nitrogen, uric acid, chlorids, purin bases, alkaline and earthy phosphates and preformed and ethereal sulphates without finding any marked changes. Their results as regards the isolation of a toxic substance from the feces could not be confirmed by Mitchell, Flexner and Edsall. The last-mentioned observers studied the amounts of ammonia and creatinin in the urine and seemed to think that there existed some relation between the attacks and the amount of creatinin excreted. They thought that the creatinin was an index of certain alterations in metabolism which leads up to a seizure and concluded that the attacks are due to some metabolic disturbance situated in the muscles.

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[8] Singer, H. D., and Goodbody, F. W.: A Case of Family Periodic Paralysis, with a Critical Digest of the Literature, Brain, 1901, xxiv, 257.

[9] Crafts and Irwin: Am. Jour. Med. Sc., 1900, cxix, 651.

TABLE 2.—The Sulphur Metabolism—

Day	Sulphur gm.	Urine			
		Total Sulphate Sulphur		Inorganic Sulphate Sulphur	
		Gm.	Percentage of Total Sulphur	Gm.	Percentage of Total Sulphur
1	1.12	1.05	93.8	0.89	79.5
2	1.29	1.19	92.3	1.08	83.8
3	1.38	1.27	92.0	0.96	69.5
4	1.30	1.18	90.8	0.88	67.8
5	1.24	1.15	92.8	1.03	83.1
6	1.00	0.85	85.0	0.74	74.0
7	1.22	1.10	90.2	1.00	82.0

METABOLIC DATA

The patient was placed on Folin's[10] diet, consisting of:

Whole milk	500 c.c.
Cream	300 c.c.
Horlick's Malted Milk	200 gm.
Sugar	20 gm.
Salt	6 gm.
Eggs	450 gm.
Water	2,000 c.c.

This diet contains approximately 119 gm. of protein, 148 gm. of fat and 225 gm. of carbohydrate, yielding 2,787 calories, or about 46 calories per kilogram body weight of the patient.

Two ounces of the foregoing diet was taken out daily and the following constituents estimated: total nitrogen, phosphorus, sulphur, calcium and magnesium. The fat was estimated in a seven-day sample, 5 c.c. of each day's mixture being preserved by means of 2 drops of liquor formaldehydi.

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[10] Folin: Am. Jour. Physiol., 1905, xiii, 45.

A. *Method Used in Urine Analysis.*—The nitrogen was estimated according to Kjeldahl, the total sulphur by Benedict's[11] method. Total and ethereal sulphates by Folin's[12] method; the inorganic sulphates were computed by subtracting the ethereal sulphates from the total sulphates, and the neutral sulphur by subtracting the total sulphate sulphur from the total sulphur. Urea was estimated by Benedict's[13] method; ammonia by Folin's[14] method; total phosphorus by the Neumann[15] method, weighing the phosphorus as magnesium pyrophosphate; creatinin and creatin by Folin's[16] method; uric acid by Folin's[17] method; calcium and magnesium by McCrudden's[18] method, and total acidity by Folin's[19] method.

—and Urinary Sulphur Partition

				Feces	Sulphur	
Ethereal Sulphate Sulphur		Neutral Sulphur		Sulphur	Intake	Balance
Gm.	Percentage of Total Sulphur	Gm.	Percentage of Total Sulphur	gm.	gm.	gm.
0.16	14.3	0.07	6.2	0.32	1.60	+ 0.16
0.11	8.5	0.10	7.7	0.32	1.70	+ 0.09
0.31	22.5	0.11	7.9	0.34	1.80	+ 0.08
0.30	23.0	0.12	9.2	0.34	1.50	— 0.14
0.12	9.7	0.09	7.3	0.30	1.40	— 0.14
0.11	11.0	0.15	15.0	0.30	1.30	0.0
0.10	8.2	0.12	9.9	0.30	1.32	— 0.22

B. *Methods Used in Analysis of Food.*—The nitrogen was estimated by the Kjeldahl method; total sulphur by the Wolf and Osterberg[20] modification of Benedict's method; calcium and magnesium by McCrudden's method after ashing and extracting the ash with hydrochloric acid, and phosphorus by Neumann's method. Fat was estimated in a five-day period by Soxhlet extraction.

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[11] Benedict: Jour. Biol. Chem., 1909, vi, 363.

[12] Folin: Am. Jour. Physiol., 1905, xiii, 51; Jour. Biol. Chem., 1906, 1, 131.

[13] Benedict: Jour. Biol. Chem., 1911, viii, 405.

[14] Folin: Ztschr. f. physiol. Chem., 1902, xxxvii, 161; Am. Jour. Physiol., 1903, viii, 330.

[15] Neumann: Ztschr. f. physiol. Chem., 1902-1903, xxxvii, 129; 1904-1905, xliii, 35.

[16] Folin: Am. Jour. Physiol., 1905, xiii, 45.

[17] Folin: Ztschr. f. physiol. Chem., 1901, xxxii, 552.

[18] McCrudden: Jour. Biol. Chem., 1911, x, 187.

[19] Folin: Am. Jour. Physiol., 1903, p. 265.

[20] Wolf and Osterberg: Biochem. Ztschr., 1910, xxix, 429.

C. *Methods Used in Analysis of Feces.*—The feces were marked off by means of carmin into periods of four and three days, respectively. Nitrogen was estimated by the Kjeldahl method; sulphur by oxidizing with fuming nitric acid followed by Benedict's method; phosphorus by the Neumann method; calcium and magnesium by the same method as used for food, and fat by Soxhlet extraction.[21]

The accompanying tables contain the results obtained in this study.

COMMENT ON TABLE 1

In the seven-day metabolism period there was a loss of 5.81 gm. of nitrogen. The absorption of nitrogen is normal. The amount of nitrogen excreted as urea and uric acid is also normal. The creatinin nitrogen in gram and creatinin nitrogen in per cent. of the total nitrogen is markedly decreased as is also the creatinin coefficient. This may indicate a poor muscular efficiency of the individual, as it will be recalled that Shaffer finds the normal creatinin coefficient to be from 7 to 11 mg. of creatinin nitrogen per kilogram of body weight and thinks that it is an index of some special process of normal metabolism, taking place largely, if not wholly, in the muscles. On the other hand, it may indicate, as Mitchell, Flexner and Edsall thought, an index of certain alterations in the metabolism which lead up to a seizure, and this disturbance is situated in the muscles.

TABLE 3.—The Calcium, Magnesium—

Day	Urine			Feces		
	Phosphorus gm.	Calcium Oxid gm.	Magnesium Oxid gm.	Phosphorus gm.	Calcium Oxid gm.	Magnesium Oxid gm.
1	1.30	0.82	0.32	1.02	1.46	0.12
2	1.32	0.79	0.28	1.02	1.46	0.12
3	1.41	0.81	0.26	1.02	1.46	0.12
4	1.46	0.84	0.24	1.02	1.46	0.12
5	1.41	0.76	0.30	1.16	1.50	0.11
6	1.52	0.78	0.32	1.16	1.50	0.11
7	1.54	0.72	0.30	1.16	1.50	0.11

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[21] In addition to the references already given, the following will be found of interest:

Buzzard, E. Farquhar: Three Cases of Family Periodic Parlysis, Lancet, London, 1901, ii, 1564.

Atwood: C. E.: New York State Med. Jour., October, 1912.

The amount of undetermined nitrogen excreted in the urine is also increased. Our knowledge of this group of nitrogenous products is very meager. We do know that the so-called neutral sulphur and colloidal nitrogen are represented in this fraction. It would be of great interest to follow the amount of undetermined nitrogen excreted in the urine during an attack. It is possible that individuals affected with periodic paralysis may be unable to metabolize those products sufficiently and therefore an increased amount is excreted in the urine, or that they produce an excessive amount of these substances and that the attack is due to retention of these products.

COMMENT ON TABLE 2

In the seven-day metabolism period there was a loss of 0.17 gm. of sulphur. The urinary sulphur partition (total sulphate sulphur, inorganic sulphate sulphur, ethereal sulphate sulphur and neutral sulphur) is normal in character. The fact that the neutral sulphur is not increased shows that this type of substance is not increased in the partition of the urine known as undetermined nitrogen. There were marked increased amounts of ethereal sulphates excreted on the third and fourth day coinciding with a period of constipation which we often find in patients placed on this diet.

—and Phosphorus Metabolism

Intake			Balance		
Phosphorus gm.	Calcium Oxid, gm.	Magnesium Oxid, gm.	Phosphorus gm.	Calcium Oxid, gm.	Magnesium Oxid, gm.
2.4	2.6	0.42	+ 0.08	+ 0.32	— 0.02
2.4	2.6	0.42	+ 0.06	+ 0.35	+ 0.02
2.4	2.6	0.42	— 0.03	+ 0.33	+ 0.04
2.4	2.6	0.47	— 0.08	+ 0.30	+ 0.11
2.2	2.1	0.42	— 0.37	— 0.16	+ 0.01
2.2	2.3	0.44	— 0.48	+0.05	+ 0.01
2.2	2.2	0.43	— 0.50	— 0.02	+ 0.02

COMMENTS ON TABLES 3 AND 4

In the seven days there was a loss of 1.32 gm. of phosphorus, a retention of 1.17 gm. of calcium oxid and 0.19 gm. of magnesium oxid. The fat metabolism is normal with an absorption of 92.8 per cent. of the ingested fat, and the percentage of neutral fat, fatty acids and soaps in the feces fat is normal.

SUMMARY

1. The clinical history and a metabolism study of seven days' duration on the Folin diet is described in a case of family periodic paralysis.

2. We have studied the nitrogen metabolism and urinary nitrogen partition (total nitrogen, urea nitrogen, ammonia nitrogen, uric acid nitrogen, creatinin nitrogen and undetermined nitrogen), the sulphur metabolism and urinary sulphur partition (total sulphates, inorganic sulphates, ethereal sulphates and neutral sulphur). We have also studied the calcium, magnesium, phosphorus and fat metabolism.

TABLE 4.—The Fat Metabolism

Period Days	Fat Intake gm.	Feces					Balance gm.
		Fat gm.	Percentage of Fat Absorbed	Percentage of Neutral Fat in Feces Fat	Percentage of Fatty Acid in Feces Fat	Percentage of Soaps in Feces Fat	
7	978	70	92.8	38	24	38	908

3. The only decided metabolic changes found was a marked decrease in the amount of creatinin and creatinin nitrogen excreted in the urine and a marked increase in the amount of undetermined nitrogen of the urine.

4. It would be of great value, using the same methods described in this paper, to repeat this study on a patient suffering from family periodic paralysis during an attack, so as to correlate the findings with those described in this paper.

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NOTE ON THE INCIDENCE OF STATUS LYMPHATICUS IN DEMENTIA PRAECOX *

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In a study of the clinical diagnosis of status lymphaticus based on the physical findings in 1,000 male patients in the alcoholic wards of Bellevue Hospital in New York, I noted[1] that among the patients referred to these wards were frequently to be found border-line cases, which on further study were properly classed as psychopathic cases instead of individuals of normal mentality who were toxic and irresponsible as a result of narcotic or alcoholic drug addictions.

Among these psychopathic patients, cases of dementia praecox were common, and in seeking an opportunity to study a larger group of such cases with a view to determining the incidence of status lymphaticus, I obtained permission through the courtesy of Dr. Wm. Mabon and the resident physicians at the State Asylum at Ward's Island to examine such inmates as were definitely classed as having dementia praecox.

No cases of doubtful diagnoses were considered, and in most instances the patients examined had been inmates for some years, and in no instance was a recent admission (within one month) classified and examined as dementia praecox.

In determining the diagnosis of status lymphaticus, I used the same criteria as I considered sufficient in the study above referred to, namely, in males, "a decided scantiness of hair on chin and upper lip, scanty axillary and sternal hair, scanty or feminine distribution of pubic hair, a slender thorax, rounded contour of upper arms and thighs, with an arching of the latter, hypoplastic external genitals, particularly if associated with cryptorchidism and a delicate velvety skin. The diagnosis is further confirmed if we find hyperplasia of the lymph tissue of nose, throat and tongue and an increase in the palpable cervical and axillary lymph-nodes."

I examined 208 men from 16 to 50 years of age, among whom sixty-one were found to exhibit the typical physical characteristics of status lymphaticus (29.3 per cent.); 147 were found to be physically normal (70.6 per cent.).

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* Submitted for publication Nov. 24, 1914.

[1] Emerson, Haven: The Archives Int. Med., 1914, xiii, 169.

Of the 61 status lymphaticus cases 9 were between 16 and 19 years of age; 27 were between 20 and 29; 15 were between 30 and 39; 8 were between 40 and 49; 2 were 50 years of age.

In the article already referred to, I ventured the unsupported statement that the incidence of status lymphaticus in cases of acute alienation in the first three decades was higher than among alcoholic and narcotic drug habitués, in whom I found an incidence of 22 per cent. This impression is now confirmed by the present report.

120 East Sixty-Second Street.

ARTIFICIAL PNEUMOTHORAX

WITH REPORT OF GAS ANALYSES AND EXPERIMENTS TO DETERMINE THE USE OF AIR OR NITROGEN [*]

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The present report will deal first with our experiences and results with artificial pneumothorax, and secondly with a series of gas analyses and animal experiments in regard to the use of air or nitrogen.

During the past two years the attempt has been made to produce an artificial pneumothorax in eighty-three patients with advanced pulmonary tuberculosis. In two cases the method of Brauer was attempted, in all others that of Forlanini.

The apparatus and method of procedure were practically similar to those so well described by Robinson and Floyd,[1] and by Hamman and Sloan;[2] therefore it will not be necessary to give the details here.

For the most part atmospheric air passed through sterile cotton filters has been the gas used. A series of cases, however, was treated with nitrogen for purposes of comparison, as will be shown in the latter half of this paper. Epinephrin (adrenalin, P. D. & Co.) and novocain were used in producing local anesthesia.

For convenience of analysis, the cases may be divided into the following groups:

I. Complete failure to find the pleural space (owing to extensive adhesions)		21 cases
II. Advanced cases, usually with cavity. Involvement of other lung not serious		25 cases
1. Remarkable benefit, loss fever, sputum, cough, etc.	18	
2. Some general benefit	6	
3. Very slight benefit	1	
4. No benefit whatever	0	
III. Far advanced; other lung involvement more serious		29 cases
1. Great benefit	7	
2. Fair degree of benefit	5	
3. Very slight benefit	8	
4. No benefit whatever	9	
IV. For hemorrhages		3 cases
1. Hemorrhages ceased but patient died later of other complications.		

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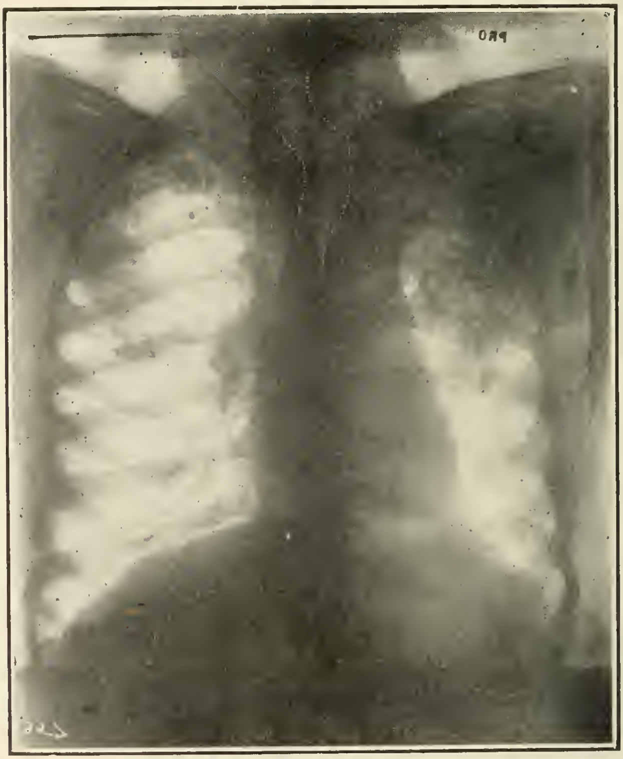
[*] Submitted for publication June 19, 1914.

[1] Robinson, Samuel, and Floyd, Cleaveland: Artificial Pneumothorax as a Treatment of Pulmonary Tuberculosis, The Archives Int. Med., 1912, ix, 453.

[2] Hamman, L., and Sloan, M. F.: Induced Pneumothorax in the Treatment of Pulmonary Disease, Johns Hopkins Hosp. Bull., 1913, xxiv, 53.

2. Completely successful.	
3. Hemorrhages ceased although very little collapse was obtained.	
V. Lung abscesses (secondary to foreign body)	2 cases
1. Apparent complete cure.	
2. Death due to bad general condition.	
VI. Lung tumor (for diagnosis and relief of pain)	1 case
VII. Brauer method used after failure of Forlanini method	2 cases
1. No benefit.	
2. Some slight benefit. Collapse in each case only partial.	



Retraction of the trachea to one side by apical fibrosis. For emphasis the trachea has been outlined by the radiologist, Dr. L. G. Brown. Fifteen years ago one of us had made a diagnosis of a dry cavity at the left apex. When two years ago the patient again came under observation the unusual position of the trachea was discovered.

The following incidents in this series of cases would seem of interest and importance:

1. In two cases which were considered for pneumothorax treatment, the Roentgen-ray examination revealed a mistake in the physical

diagnosis. In each case what had been considered an apical cavity was shown on the plates to be the trachea pulled to one side by apical fibrosis. (See illustration.)

2. Retraction of the trachea to one side may signify adhesions at the apex which will prevent collapse of that part of the lung should the remedy of pneumothorax be applied.

3. Within twenty-four hours of the first application of pneumothorax, spontaneous and complete pneumothorax followed in three patients, one in Class II and two in Class III. In no case, however, did this accident result fatally, although for a short time dangerous and alarming symptoms were present. We feel that this is a possible danger hitherto not sufficiently emphasized.

4. In the series the left lung much more frequently needed collapsing than the right.

5. Three brothers had their left lungs successfully collapsed.

6. In one patient with severe paroxysmal coughing the left lung was collapsed, with the exception of the cavity, which was located in the lower part of the upper lobe.

7. This patient later broke two ribs on the collapsed side as a result of his violent cough.

8. All cases should be handled at first like typhoid cases.

9. Over 900 injections of gas or air have been safely given.

10. No case of so-called pleural reflex has been encountered.

11. A slight case of air embolism probably occurred once. During a refilling, the patient turned slightly to observe the manometer. Unconsciousness and stertorous breathing occurred for a few moments. The next day a transient weakness of one hand was noticed.

12. An appreciable amount of fluid developed in ten cases, or 16 per cent. This was partly withdrawn in several cases on several occasions and usually lessened or disappeared. The fluid appeared late in the treatment.

13. The fluid became purulent, but culturally sterile, in three of these cases. Two of these were patients in whom the pneumothorax failed to give benefit and had been given up.

14. Two patients who refused the application of pneumothorax, and whose conditions seemed hopeless without attempting this remedy, and two in whom we were unable to induce it, are now in excellent condition, walking several miles a day, and with almost complete absence of expectoration.

15. Conclusions in regard to the amount of collapse of the affected lung can frequently be drawn by careful attention to the amount of sputum, the temperature, the pulse and the physical examination, with-

out the employment of the Roentgen ray. The Roentgen ray has been of scarcely any value in predicting the absence of adhesions or the possibility of collapse.

16. Experience has shown us that the compression should be kept up for probably several years. One patient after apparently complete recovery for two years began to have fever again as a result of too infrequent recompression. Hemorrhages recurred eight months later in a patient included in Group IV, who had only allowed the compression to be kept up three months.

CONCLUSIONS

The procedure of artificial pneumothorax is of undoubted value in many advanced cases of pulmonary tuberculosis. The dangers in its application with proper precautions would seem negligible, except as stated in Paragraph 3 above. The necessity for keeping up the compression over a long period of time is a serious drawback, as is also the possible annoyance to the patient of the development of fluid. For the present every known method should be very thoroughly tried out before suggesting pneumothorax to our patients.

In May, 1912, J. S. Haldane, the Oxford physiologist, expressed the opinion to one of us that the use of nitrogen, as advocated for artificial pneumothorax, could have little, if any, advantage over the use of air, because in either case diffusion of gases would so rapidly occur.

Little can be found in the literature regarding the analyses of the gaseous contents of the chest in cases of artificial pneumothorax and the employment of nitrogen instead of atmospheric air would seem to be based on theoretical rather than experimental grounds.

Wells[3] states that when air enters a closed pleural cavity, and no effusion follows, it is slowly absorbed. At first there is a rapid absorption of oxygen which is partly replaced by carbon dioxid with a resulting relative increase in nitrogen. Ordinarily, the entrance of air into the pleural cavity is followed by an effusion either serous or purulent which may modify the composition of the gases. Purulent pneumothorax generally shows more carbon dioxid than the serous form.

Hamman[2] states that the normal pleural cavity absorbs from 80 to 100 c.c. nitrogen per day; after the pneumothorax has existed for some months it absorbs from 25 to 50 c.c.

Tobiesen[4] has published a few gas analyses, and in a very interesting paper has given a short historical sketch which we briefly abstract.

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[3] Wells: Chemical Pathology.

[4] Tobiesen, T.: Untersuchungen über Pneumothorax luft, Beitr. z. Klin. d. Tuberk., 1911, xix, 451; ibid., 1911, xxi, 109.

In 1805 Itart described the pathological condition of pneumothorax and gave it the present name.

In 1823 Davy for the first time analyzed pneumothorax air from a patient. He also injected air into the pleural cavity of animals and then withdrew samples and analyzed them. He found that oxygen became absorbed and that carbon dioxid diffused out into the pleural space.

Demarquay and Leconte were able to decide by gas examinations whether spontaneous pneumothorax cases were "open" or "closed." They state that nitrogen is slowly absorbed by the pleural wall.

Tobiesen reports sixteen gas analyses in seven cases of spontaneous pneumothorax, five analyses in three cases of artificial pneumothorax induced by atmospheric air, and six analyses in three cases induced by nitrogen gas.

The nitrogen gas employed yielded the following analysis: oxygen 4.31 per cent., carbon dioxid 0.62 per cent. and nitrogen 95.07 per cent.

Table 1 depicts Tobiesen's results.

TABLE 1.—Results of Tobiesen's Gas Analysis in Cases of Pneumothorax, Natural and Artificial

Case No.	Total Air Injected	Days Since Injected	Amount at Last Injection	O_2	Per Cent. CO_2	N
8	1. 2,850	8	1,000	2.48	6.32	91.20
	2. 4,650	7	800	2.86	....	90.62
9	1. 2,450	6	800	0.95	7.04	92.01
10	1. 2,600	6	500	0.71	8.32	90.97
	2. 4,300	14	600	0.69	9.31	90.00
	Total Nitrogen Injected					
11	1. 4,200	7	300	0.09	10.26	89.65
	2. 4,500	9	300	0.05	10.12	89.83
12	1. ?	14	700	2.67	7.15	90.18
	2.	14	500	2.82	6.98	90.20
13	1. 1,260	3	320	1.96	7.14	90.90
	2. 2,060	5	400	1.66	7.39	90.95

On analyzing these figures little difference can be detected in favor of nitrogen; in fact, the amount of nitrogen determined in the cases in which air had been employed reads the higher.

It seemed to us worth while, therefore, especially in consideration of Haldane's unusual knowledge regarding the lungs and gaseous diffusion, to carry out a series of analyses with patients undergoing treatment by the application of artificial pneumothorax.

From the first it was realized that it would be impossible to regain and measure the whole amount of gas injected in patients and the final proof, therefore, of any advantage of nitrogen gas over atmospheric air might be lacking. We foresaw that simple analyses of the samples withdrawn from the pleural space could yield only indirect and incomplete evidence.

The first gas analyses were made with samples obtained from a large dog in which artificial pneuthorax had been induced with nitrogen withdrawn from a cylinder obtained from a commercial house.

The results[5] of the analysis of this so-called pure nitrogen obtained directly from the cylinder were startling: oxygen 13.98 per cent., carbon dioxid none and nitrogen 86.02 per cent.

TABLE 2.—Experiment on Large Dog

Date	Gas Removed c.c.	Yielding Per Cent.			Gas Injected c.c.
		O_2	CO_2	N	
3/23/13	....	....	....	....	400
3/24/13	30	14.5	Trace	85.5	500
3/25/13	35	15.06	3.74	81.2	700
3/28/13	35	10.42	5.70	83.88	

This experiment clearly showed that carbon dioxid was gradually diffused out into pleural space.

We now decided to manufacture our own nitrogen, and the following method was employed, a method which is convenient, cheap and productive of gas in a very pure form: two parts of a 40 per cent. solution of sodium nitrite are mixed with three parts each of a 20 per cent. solution of ammonium sulphate and a 20 per cent. solution of potassium dichromate. This is heated gently and passed through a potassium pyrogallate solution and through sulphuric acid and collected by displacement of water. As will be seen by the tables, we were enabled to procure, by this means, nitrogen about 99 per cent. pure.

In a few cases we used oxygen for the production of pneumothorax. The method used for preparing oxygen was to mix four parts potassium chlorate with one part manganese oxid. This is heated slowly in a test-tube and collected by displacement of water.

A series of patients treated with nitrogen were now compared with a series of patients treated with air. The method of procedure was as follows:

The samples of the gas mixtures were always withdrawn from the thorax before introducing a fresh supply. The needle having been inserted as usual and the oscillations obtained, the tube to the bottle containing the air, or gas, to be injected was clamped off close to the needle, and the gas-sampling tube attached to the other projection of the needle, which could be opened by a stop-cock. The sampling tube had a capacity of about 30 c.c. and was provided at each end with a two-way stop-cock. The tube was previously completely filled with mercury and the gas drawn from the pleural cavity into the gas receiver by allowing the mercury to escape through the lower end of the tube.

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[5] Haldane and his co-workers had a similar experience with commercial oxygen during their expedition to Pike's Peak in 1911.

The sample thus obtained was analyzed with the smaller Haldane gas analysis apparatus.[6] This is essentially as follows: The sample is drawn by mercury suction into a graduated buret and the amount of gas read. By means of mercury the gas is now forced over a connecting tube into a bulb of 15 per cent. caustic potash which absorbs the carbon dioxid. The difference is now read on the graduated buret and the percentage of carbon dioxid thus obtained. The gas is then run over into a bulb containing a 10 per cent. solution of potassium pyrogallate, which absorbs the oxygen.

The difference between the amount left after absorption of carbon dioxid and the amount now left is the quantity of oxygen in the sample.

For all practical purposes the remaining gas may be considered nitrogen.

Tables 3 to 8 typify individual cases treated with nitrogen gas and with atmospheric air. These tables would indicate that within from twenty-four to forty-eight hours, in the nitrogen-treated cases, about 4 per cent. oxygen and about 8 per cent. carbon dioxid appear in the pleural space, or, in other words, the nitrogen becomes diluted at least 10 per cent. If the law of diffusion of gases holds good in these cases, then some of the pure nitrogen must diffuse in the opposite direction into the gases of the lungs tending to establish equilibrium.

When atmospheric air is injected, within a similar period of time practically exactly similar analyses are obtained as in the nitrogen cases. Therefore, diffusion of oxygen to at least 15 per cent. has taken place into the blood. On the other hand, diffusion of carbon dioxid to about 7 per cent. from the blood and of nitrogen to about 10 per cent. from the lung alveolar air has occurred into the pleural space. The treated lung being collapsed and compressed, equilibrium is probably established through the mediastinal pleura from the alveolar air of the opposite lung. In fact, the percentages in the two types of cases run parallel and no essential difference can be noted. Those who analyzed the gas sample could not at any stage tell whether the patient had been treated with pure nitrogen or with pure air.

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[6] Described in Haldane's "Methods of Air Analysis." London, Charles Griffin & Co., ltd., 1912.

TABLE 3.—Case 51, Treated with Nitrogen Gas

Date	Injection No.	Amount Given	Analysis of Gas Removed			Analysis of Gas Given			Pressure in Cm. Water	
			O_2	CO_2	N	O_2	CO_2	N	Before	After
9/20/13	1	400	....	....	.....	4.25	1.64	94.11	—2	—2
9/22/13	2	600	8.66	6.55	84.79	0.15	0.52	99.33	—2	—1
9/24/13	3	700	4.08	6.74	88.58	0.15	0.92	98.92	0	+1
9/29/13	4	800	3.08	7.26	89.66	0.1	0.61	99.29	0	+2
10/ 6/13	5	300	1.91	8.11	89.98	0.31	0.13	98.56	0	+2
10/15/13	6	400	1.31	9.02	89.67	2.75	0.41	96.84	0	+3
10/31/13	7	800	1.52	8.01	90.47	....	....	.....	0	+4

TABLE 4.—Case 47, Treated with Atmospheric Air

Date	Injection No.	Amount Given	Analysis of Gas Removed			Analysis of Gas Given			Pressure in Cm. Water	
			O_2	CO_2	N	O_2	CO_2	N	Before	After
7/16/13	1	200	....	....	.....	20.93	0.03	79.04	—2	—2
7/17/13	2	300	7.92	6.85	85.23	....	....	.....	—3	0
7/18/13	3	500	6.64	7.82	85.54	....	....	.....	—2	0
7/21/13	4	800	4.17	7.08	88.75	....	....	.....	—2	+1
7/26/13	5	800	2.15	7.83	90.02	....	....	.....	—1	+1
8/ 2/13	6	1,000	2.31	7.47	90.22	....	....	.....	—0	+2
8/11/13	7	1,100	1.97	8.00	90.03	....	....	.....	—1	+3
8/21/13	8	1,250	2.28	8.20	89.52	....	....	.....	—1	+4
9/ 2/13	9	1,250	1.86	8.33	89.81	....	....	.....	0	+5
9/16/13	10	1,400	1.58	8.28	90.14	....	....	.....	—1	+4
10/ 6/13	11	1,200	2.35	8.70	88.95	....	....	.....	0	+5

TABLE 5.—Case 52, Treated with Nitrogen Gas

Date	Injection No.	Amount Given	Analysis of Gas Removed			Analysis of Gas Given			Pressure in Cm. Water	
			O_2	CO_2	N	O_2	CO_2	N	Before	After
10/14/13	1	500	....	....	.....	0.61	0.72	98.67	— 2	0
10/18/13	2	600	4.84	7.33	87.83	0.10	0.82	99.08	— 2	—2
10/22/13	3	700	4.81	6.57	88.62	....	....	.....	— 1	+4
10/28/13	4	600	....	....	.....	....	....	.....	0	+4
11/ 2/13	5	700	3.67	5.96	90.37	....	....	.....	— 1	+4
11/ 7/13	6	1,200	3.75	7.11	89.14	....	....	.....	—½	+7
11/12/13	7	600	....	....	.....	....	....	.....	+ 1	+7
11/17/13	8	500	3.70	6.41	89.89	....	....	.....	+ 4	+9
11/24/13	9	600	4.39	6.65	88.96	....	....	.....	+ 3	+7
12/ 6/13	10	800	3.97	6.16	89.87	....	....	.....	+ 2	+7

TABLE 6.—CASE 44, TREATED WITH ATMOSPHERIC AIR

Date	Injection No.	Amount Given	Analysis of Gas Removed			Analysis of Gas Given			Pressure in Cm. Water	
			O_2	CO_2	N	O_2	CO_2	N	Before	After
6/30/13	1	350	....	....	.....	20.93	0.03	79.04	—3	—3
7/ 2/13	2	200	5.63	6.18	88.19	....	....	.....	0	+1
7/ 5/13	3	400	4.66	6.07	89.27	....	....	.....	—1	+2
7/ 9/13	4	400	3.07	7.23	89.70	....	....	.....	0	+3
7/15/13	5	600	2.09	7.63	90.28	....	....	.....	—1	+3
7/22/13	6	650	2.12	8.16	89.72	....	....	.....	—1	+4
7/30/13	7	600	2.79	7.84	89.87	....	....	.....	—1	+3
8/ 8/13	8	700	2.86	8.29	88.85	....	....	.....	—1	+4
8/18/13	9	600	1.45	8.83	89.72	....	....	.....	—2	+3
8/28/13	10	650	1.80	8.84	89.36	....	....	.....	—1	+4
9/11/13	11	650	1.67	8.79	89.54	....	....	.....	—1	+4

TABLE 7.—CASE 46, TREATED WITH ATMOSPHERIC AIR

Date	Injection No.	Amount Given	Analysis of Gas Removed			Analysis of Gas Given			Pressure in Cm. Water	
			O_2	CO_2	N	O_2	CO_2	N	Before	After
7/15/13	1	150	....	....	.....	20.93	0.03	79.04	—2	—2
7/17/13	2	200	5.07	8.44	86.49	....	....	.....	—2	0
7/19/13	3	300	....	....	.....	....	....	.....	—2	0
7/22/13	4	450	3.85	6.63	89.52	....	....	.....	—1	—1
7/26/13	5	500	....	....	.....	....	....	.....	—1	—3
8/ 2/13	6	600	2.55	7.60	89.85	....	....	.....	—1	—5
8/11/13	7	900	2.22	7.51	90.27	....	....	.....	—1	—6
8/21/13	8	800	2.60	2.85	90.55	....	....	.....	—1	—5

TABLE 8.—CASE 54, TREATED WITH ATMOSPHERIC AIR

Date	Injection No.	Amount Given	Analysis of Gas Removed			Analysis of Gas Given			Pressure in Cm. Water	
			O_2	CO_2	N	O_2	CO_2	N	Before	After
10/15/13	1	300	....	....	.....	2.75	0.61	96.64	—6	—2
10/17/13	2	500	2.44	7.15	90.41	....	....	.....	—4	—2
10/21/13	3	800	....	....	.....	....	....	.....	—2	—2
	4	800	1.46	9.56	88.98	....	....	.....	—2	—2
	5	750	0.80	10.03	89.17	....	....	.....	—3	—3
	6	900	0.70	10.53	88.77	....	....	.....	0	—5
	7	1,000	0.71	9.85	89.44	....	....	.....	—2	—6
	8	800	1.11	9.61	89.28	....	....	.....	—2	—6

TABLE 9.—Nitrogen Cases

No. of Case	Date	No. Injection	Amount Given to Date in c.c.	Days Since Last Injection	Amt. Given in c.c.	Gas Analysis			Pressure	
						CO_2	O_2	N	Before	After
50	8/16/13	..	....	15	800	8.36	3.96	87.68	—2	— 5
50	8/27/13	..	....	11	1,000	7.53	3.05	89.42	—3	— 9
50	9/12/13	..	....	16	950	7.97	4.73	87.50	—2	— 8
51	9/22/13	2	400	2	600	6.55	8.66	84.79	—2	0
51	9/24/13	3	1,000	2	700	6.74	4.68	88.58	0	— 1
50	9/27/13	..	....	15	900	7.98	4.61	87.41	0	— 8
50	10/ 3/13	..	....	6	800	7.74	5.06	87.20	—1	— 9
51	9/29/13	4	1,700	5	800	7.26	3.08	.76	0	— 2
51	10/ 6/13	5	2,500	8	300	8.11	1.91	.....	0	— 2
51	10/15/13	6	2,800	9	400	9.02	1.31	.....	0	— 3
54	10/17/13	2	300	2	500	7.10	2.44	.....	—4	— 2
52	10/18/13	3	800	4	600	7.33	4.84	87.83	—2	— 2
52	10/22/13	4	1,400	4	700	6.57	4.81	.....	—1	— 4
54	10/28/13	4	....	7	800	9.56	1.46	88.98	—2	— 2
53	10/29/13	3	1,000	7	800	6.54	4.98	.....	—2	— 3
51	10/31/13	7	3,200	16	800	8.01	1.52	90.47	0	— 4
52	11/ 2/13	6	2,700	5	700	5.92	3.67	90.37	—1	— 5
54	11/ 3/13	5	2,400	6	750	10.03	0.80	89.17	—1	— 3
52	11/ 7/13	7	3,400	5	1,200	7.11	3.75	89.14	—1	— 7
53	11/ 7/13	4	1,800	9	900	6.66	4.50	88.84	—2	— 5
50	11/ 7/13	..	....	19	900	7.80	5.05	87.15	0	— 8
54	11/ 8/13	6	3,150	5	900	10.53	0.70	88.77	0	— 5
54	11/13/13	7	4.050	5	1,000	9.85	0.71	89.44	—2	— 6
52	11/17/13	9	5,200	5	500	6.41	3.70	89.89	—4	— 9
53	11/17/13	5	2,700	10	1,000	4.51	7.09	89.40	—3	—10
54	11/22/13	8	5,050	9	800	9.61	1.11	89.28	—2	— 6
52	11/24/13	10	5,700	7	600	6.65	4.39	88.96	—3	— 7
53	11/29/13	6	3,700	12	650	6.86	5.22	87.92	0	— 7
50	11/29/13	..	....	22	650	8.05	4.82	87.13	—1	— 8
52	12/ 6/13	11	6,300	12	800	6.16	3.97	89.87	—2	— 7
(a)	6/29/13	9	4,215	6	350	10.87	1.10	88.03		
(b)	7/ 9/13	19	10.445	35	125	12.40	0.419	87.19		
(c)	7/ 9/13	5	1,500	4	580	6.81	2.37	90.82		
(a)	7/28/13	12	5,315	12	300	10.59	1.02	88.39		
(c)	7/28/13	19	3,280	5	...	7.28	2.34	90.38		
(d)	8/20/13	4	1,000	6	250	7.43	5.51	87.06		
(a)	8/20/13	15	6,715	10	400	11.08	*	88.92		
(b)	10/ 8/13	21	10,660	60	...	11.21	2.58	86.21		
(a)	10/ 8/13	19	7,965	18	500	10.06	1.73	88.21		

* Trace.

TABLE 10.—ATMOSPHERIC AIR CASES

No. of Case	Date	No. Injection	Amount Given to Date in c.c.	Days Since Last Injection	Amt. Given in c.c.	Gas Analysis			Pressure	
						CO_2	O_2	N	Before	After
26	6/21/13	13	112,000	21	850	6.80	4.51	88.69	—1	— 5
37	6/23/13	5	2,050	15	900	6.35	4.34	89.31	—1	— 2
34	6/23/13	7	4,850	15	800	6.42	4.23	89.35	0	— 4
42	6/24/13	5	1,800	7	600	6.61	3.46	89.93	—1	— 2
40	6/25/13	7	3,700	12	900	8.71	1.40	89.89	0	— 5
13	6/26/13	14	113,000	10	1,000	5.31	7.66	87.03	—8	— 8
41	6/26/13	5	2,100	23	700	7.47	4.25	88.28	—1	— 4
36	6/27/13	7	3,550	11	600	8.65	1.69	89.66	—1	— 3
19	6/28/13	15	....	25	350	9.56	1.39	89.05	—1	—10
27	6/30/13	14	7,000	23	300	6.87	2.09	91.04	—1	—12
14	6/30/13	19	10,500	28	300	6.95	4.51	88.54	—2	—15
21	6/30/13	12	11,800	20	1,100	6.73	3.73	89.54	—2	— 5
44	7/ 2/13	2	350	2	200	6.18	5.63	88.19	0	— 1
33	7/ 2/13	9	7,800	14	900	8.16	4.33	87.51	—1	— 5
25	7/ 3/13	11	....	37	600	10.13	0.67	89.20	—2	— 6
2	7/ 3/13	..	....	33	800	6.67	3.02	90.31	—2	—10
36	7/ 5/13	8	4.100	8	650	9.34	1.49	89.17	—2	— 8
44	7/ 5/13	3	550	3	.....	6.07	4.66	89.27	—1	— 2
40	7/ 8/13	8	5.200	13	700	9.31	1.64	89.05	0	— 5
7	7/ 8/13	15	11,800	40	650	12.59	0.89	86.52	—1	—10
22	7/ 9/13	11	7,700	39	700	13.59	1.12	85.29	—1	— 6
44	7/ 9/13	4	950	4	400	7.23	3.07	89.70	0	— 3
41	7/11/13	6	2.800	15	700	5.96	6.41	87.63	0	— 7
34	7/11/13	8	5,600	18	950	6.52	5.47	88.01	—2	— 3
43	7/12/13	..	....	23	250	12.50	0.75	86.75	—1	— 7
36	7/13/13	9	4,800	8	650	9.01	1.52	89.47	0	— 4
44	7/15/13	5	1,350	6	600	7.63	2.09	90.28	—1	— 3
26	7/14/13	14	11,900	23	1,050	7.81	3.56	88.63	—2	— 4
46	7/17/13	2	150	2	.....	5.07	8.44	86.49	—2	0
47	7/17/13	2	200	1	300	6.85	7.92	85.23	—3	0
36	7/18/13	10	5,450	5	725	6.25	3.56	90.69	—1	— 8
33	7/18/13	10	8,700	16	1,000	10.49	3.83	85.68	—1	— 7
31	7/18/13	9	6,600	28	1,000	10.57	0.56	88.87	—1	— 7
47	7/18/13	3	500	1	500	7.82	6.64	85.54	—2	0
46	7/22/13	4	650	3	450	6.63	3.85	89.52	—1	— 1
44	7/22/13	6	1,950	7	650	8.16	2.12	89.72	—1	— 4
47	7/21/12	4	1,000	3	800	7.08	4.17	88.75	—2	— 1
40	7/24/13	9	5,900	16	800	9.53	1.36	89.11	0	— 5
27	7/24/13	15	7,300	24	250	7.37	1.92	90.71	0	—14
48	7/25/13	3	600	4	600	5.67	6.70	87.63	—1	— 3
47	7/26/13	5	1,800	5	800	7.83	2.15	90.02	—1	— 1
25	7/27/13	..	....	24	700	10.39	1.37	88.24	—1	— 6
44	7/30/13	7	2,600	8	600	7.84	2.29	89.87	—1	— 3
21	7/30/13	13	12,900	31	1,100	6.46	4.55	88.99	—2	— 5
42	8/ 1/13	8	3.600	16	900	7.17	2.43	90.40	—1	— 6
46	8/ 2/13	6	1.400	7	600	7.60	2.55	89.85	—1	— 5
40	8/ 2/13	11	6.700	9	950	8.98	4.03	86.99	—1	— 5
14	8/ 2/13	20	10,800	33	500	7.61	4.62	87.77	0	—18
47	8/ 2/13	6	2,600	7	1,000	7.47	2.31	90.22	0	— 2
5	8/ 2/13	11	4,600	84	300	13.04	2.79	84.17	—6	—14
7	8/ 4/13	16	12,450	27	700	10.08	5.64	84.28	—1	—10
48	8/ 7/13	5	....	8	800	6.77	5.76	87.47	0	— 6
44	8/ 8/13	8	3.200	9	700	8.29	2.86	88.85	—1	— 4
26	8/ 9/13	15	12.950	26	1,000	8.57	2.79	88.64	—2	— 5

TABLE 10.—(Continued)

No. of Case	Date	No. Injection	Amount Given to Date in c.c.	Days Since Last Injection	Amt. Given in c.c.	Gas Analysis			Pressure	
						CO_2	O_2	N	Before	After
24	8/ 9/13	13	14,800	25	700	6.89	3.66	89.45	— 3	— 5
46	8/11/13	7	2,000	9	900	7.51	2.22	90.27	— 1	— 6
47	8/11/13	7	3,600	9	1,100	8.00	1.97	90.03	— 1	— 3
12	8/14/13	14	11,500	75	950	6.26	5.74	88.00	— 5	— 3
2	8/15/13	..	....	43	800	7.55	3.94	88.51	— 2	— 12
43	8/16/13	..	....	36	550	12.50	0.72	86.45	— 2	— 16
33	8/16/13	11	9,650	29	800	9.30	3.35	87.35	— 2	— 7
25	....	..	....	20	600	10.88	*	89.12	— 2	— 7
44	8/18/13	9	3,900	10	600	8.83	1.45	89.72	— 2	— 3
47	8/21/13	8	4,700	10	1,250	8.20	2.28	89.52	— 1	— 4
46	8/21/13	8	3,100	10	800	6.85	2.60	90.55	— 1	— 5
40	8/21/13	11	7,050	19	900	8.44	2.24	89.32	— 1	— 4
14	8/26/13	21	....	23	450	8.25	3.80	87.95	0	— 18
48	....	7	3,700	10	900	6.90	5.28	87.82	— 1	— 8
44	8/28/13	10	4,500	10	650	8.84	1.80	89.36	— 1	— 4
28	....	11	10,050	35	1,250	11.11	0.70	88.19	— 2	— 6
47	9/ 2/13	9	5,950	11	1,250	8.33	1.86	89.81	0	— 5
48	9/ 6/13	8	4,600	12	800	5.72	8.71	85.52	— 2	— 5
26	9/ 7/13	16	15,100	29	1,000	8.66	2.28	89.06	— 2	— 4
21	....	14	12,900	39	1,000	7.64	3.24	89.12	— 4	— 5
33	9/12/13	12	10,450	27	800	9.31	3.60	87.09	— 4	— 3
44	....	11	5,150	15	650	8.79	1.67	89.54	— 1	— 4
13	9/18/13	..	....	34	700	7.28	4.82	87.90	— 2	— 6
48	9/19/13	9	5,400	13	650	5.72	6.24	88.04	0	— 5
2	....	..	....	35	700	9.37	2.06	88.57	— 2	— 4
26	9/20/13	17	16,100	13	650	8.96	1.73	89.31	— 1	— 5
40	9/23/13	12	7,950	18	1,000	8.59	1.41	90.00	— 1	— 4
7	....	..	....	50	1,400	9.07	3.71	87.22	— 5	— 5
47	10/ 6/13	11	8,600	20	1,200	8.70	2.35	88.95	— 0	— 5
56	11/23/12	3	900	3	900	7.43	3.10	89.47	2	— 1
48	12/ 2/13	10	5,950	15	600	7.14	3.87	88.99	0	— 4

* Trace.

TABLE 11.—Oxygen Cases

No. of Case	Date	No. Injection	Amount Given to Date in c.c.	Days Since Last Injection	Amt. Given in c.c.	Gas Analysis			Pressure	
						CO_2	O_2	N	Before	After
58	12/ 3/13	2	500	2	900	4.16	24.04	71.80		
58	12/ 8/13	3	1,400	5	1,200	4.53	19.77	75.70	— 3	— 1
58	12/11/13	4	2,600	3	1,000	2.64	21.76	75.40	— 3	0
59	12/ 6/12	2	300	3	500	1.02	15.25	83.73		
59	12/11/13	3	800	5	500	6.52	7.41	86.07	— 5	— 2

Analysis of gas given 96.56 pure.

Clinically, the amount of gas necessary to produce and maintain a pneumothorax varied greatly in individual cases and seemed more dependent on the size of the chest, the extent of the adhesions, and the rate of diffusion through the tissues of the individual than on the nature of the gas given. On the whole, it may have seemed necessary to use a trifle less nitrogen than air to maintain a definite positive pressure in the pleural cavity. Still in one patient in whom pneumothorax was induced and kept up with nitrogen, we had to make more frequent injections to keep down her temperature and expectoration than in another patient in the same house treated with air and with similar lung involvement. Thus the rapidity of absorption in each case seems to be a law unto itself.

The oxygen cases are especially noteworthy from the discovery of the large addition of nitrogen which must have diffused into the pleural space. Since blood contains only a small percentage of nitrogen (about 2.4 per cent.), diffusion must take place between alveolar air and pleural space to establish equilibrium. It would, therefore, seem certain that whatever gas is used for the production of pneumothorax, it does not long remain pure; for Nature rapidly changes it until an analysis yields the equivalent of alveolar air. At our elevation (6,000 feet) alveolar air averages carbon dioxid 6 per cent., oxygen 14 per cent. and nitrogen 80 per cent.

In general, the longer the time between injections, the higher the percentage of carbon dioxid and the lower the percentage of oxygen, the former tending to reach 10 per cent. and the latter from 1 per cent. to zero.

For a series of crucial experiments, we performed the operation of pneumothorax on monkeys which had been used for experimental tuberculous infection.

The first result obtained follows:

Experiment 1.—The right lungs of two monkeys (*Macacus rhesus*) of equal size and weight were collapsed under ether, one with air and the other with nitrogen as the figures indicate.

	Monkey 1		Monkey 2	
Day	Air, c.c.	Pressure	Nitrogen, c.c.	Pressure
1st	45	—	45	—
2d	80	+	80	+

Analysis of nitrogen direct from generator: carbon dioxid 0.22, oxygen 0.32, nitrogen 99.46.

Analysis of nitrogen direct from apparatus used for injection: carbon dioxid 0.83, oxygen 4.89, nitrogen 94.28.

On the fourth day both monkeys were killed. The right pleural spaces were immediately filled cautiously with water through a fine needle and simultaneously the gaseous contents which escaped through another needle, were carefully collected by displacement of water.

The amount collected from No. 1 measured 83 c.c. and on analysis gave carbon dioxid 8.06, oxygen 4.24 and nitrogen 87.70; that from No. 2 measured 92 c.c. and on analysis gave carbon dioxid 8.52, oxygen 5.61 and nitrogen 85.87.

It is thus seen that forty-eight hours after positive pressure had been induced, the balance in favor of the use of nitrogen was only 7 per cent. The analysis of the gases removed is practically the same in each instance.

Experiment 2.—The same procedure was followed as in Experiment 1, but the injections were continued for a longer time so that conditions more nearly approximating the usual pneumothorax treatment would be reached.

	Monkey 7, Weight 2,700 gm.		Monkey 10, Weight 3,400 gm.	
Day	Nitrogen, c.c.	Pressure	Air, c.c.	Pressure
1st	45	—	45	—
3d	80*	—	80	—
6th	65	+(1 cm.)	65	—(1 cm.)
9th†	50	+(2 cm.)	50	—(1 cm.)
12th	70	+(2 cm.)	110	+(2 cm.)

* Analysis of nitrogen given from injection apparatus 97 per cent. pure.

† As Monkey 10 was somewhat the larger, it was decided to inject enough air next time (twelfth day) to bring the positive pressure the same in each animal.

Total nitrogen injected, No. 7, 310 c.c.
Total air injected, No. 10, 350 c.c.

Fifteenth day animals killed by chloroform. Gas contents of right pleural spaces collected as before.

	N (%)	O_2 (%)	CO_2 (%)
Monkey 7, 140 c.c. recovered, analyzing	86.74	3,57	9.69
Monkey 10, 181 c.c. recovered, analyzing	85.09	4.85	10.06

Again the gas analyses of the contents removed are practically the same in each instance. These samples were collected direct from the pleural spaces by the mercury pump method above described.

Monkey 7, of the total nitrogen injected, 46 per cent. was recovered.
Monkey 10, of the total air injected, 51 per cent. was recovered.

In all these monkeys post-mortem examination was made to determine that the technic had been accuraate.

CONCLUSIONS

We feel that the conjecture of Haldane is fully upheld by the foregoing determinations and experiments, and that little if any advantage is to be gained by the employment of nitrogen rather than atmospheric air for the production of artificial pneumothorax.

We desire to acknowledge, among the foregoing, cases kindly referred to us by Drs. Boyd, Forster, Hoagland, Martin, Timmons, Stough, Shivers and Swan. Our thanks are due to Mr. and Mrs. B. C. Allen and Mr. Richard F. Howe of Colorado Springs for generous financial support. To Dr. E. C. Schneider and to the laboratories of Colorado College we are under obligations for the facilities allowed us for the gas determinations.

Cragmor Sanatorium.

THE EFFECT OF STRYCHNIN, CAFFEIN, ATROPIN AND CAMPHOR ON THE RESPIRATION AND RESPIRATORY METABOLISM IN NORMAL HUMAN SUBJECTS *

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The effects which drugs may, conceivably, have on respiration are of four varieties.

1. They may either stimulate or depress the respiratory center.
2. They may alter respiration indirectly by increasing or decreasing metabolism.
3. They may alter the general character of breathing, rapid and shallow or slow and deep, or thoracic or abdominal by producing changes in the mechanical conditions within the chest or abdomen, by acting on the circulation, the abdominal viscera or the thoracic or abdominal musculatures.
4. They also may alter respiration by acting as bronchoconstrictors or dilators, and hence altering the dead space in the chest, which necessitates a compensatory change in the ventilation.

The data which must be obtained in studying these various actions are: the carbon dioxid elimination and the oxygen absorption, which give the metabolism; the carbon dioxid tension of the alveolar air and the ventilation of the lungs, which give information as to the excitability of the respiratory center, and the relation of ventilation to respiration rate, which gives the character of the breathing. In regard to ventilation we wish to emphasize the point, discussed in a previous communication by one of us (D. L. E.[1]), that it is the alveolar and not the total ventilation, which is of importance. The chief function of the respiratory center is to maintain a ventilation of the lung alveolae sufficient to keep the tension of carbon dioxid at a definite level. What this level shall be depends on the excitability of the center. The amount of ventilation necessary to accomplish this result depends on the elimination of carbon dioxid, and hence on the metabolism. Haldane[2] and

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[1] Edsall, D. L.: The Clinical Study of Respiration, Med. Communications, Mass. Med. Soc., 1912, xxiii, 19.

[2] Haldane, J. S., and Priestley, J. G.: The Regulation of Lung Ventilation, Jour. Physiol., 1905, xxxii, 224.

his co-workers have definitely shown that it is the tension of carbon dioxid in the arterial blood, and hence in the alveolar air (for the tension of gases in the blood leaving the lungs is always practically in equilibrium with that of the alveolar air), which regulates lung ventilation, and that the tension itself will depend on the excitability of the center. In other words, the ventilation of the lungs will always be such that the carbon dioxid tension of the alveolar air is kept at whatever level the center requires. This truth having been established, the following relationships will become apparent: First. the elimination of carbon dioxid remaining constant, changes in the alveolar ventilation will indicate changes in the excitability of the center. Secondly, the excitability of the center remaining the same, changes in the alveolar ventilation will indicate changes in the carbon dioxid elimination. Also, provided that no acidosis is present — and this assumption is reasonable in the case of normal subjects — or if present, that it remains constant during the period of observation, changes in the carbon dioxid tension of the alveolar air may always be interpreted as indicating a change in the excitability of the respiratory center.

From the foregoing remarks it will become clear, we hope, that it is the alveolar ventilation and the carbon dioxid tension, rather than the total ventilation and respiration rate, which are of chief importance, in studying the condition of the respiratory center. We wish to emphasize this point particularly, because in most previous work it has been the total ventilation and rate that have been chiefly studied.

The object of the present research has been, therefore, to investigate the action of the drugs commonly used as respiratory stimulants, bearing in mind the points brought out above, namely, that the alveolar ventilation and the carbon dioxid tension of the alveolar air are the factors which give information regarding the center, and that the total ventilation and rate may be subject to wide variation without there being any change in the excitability of the center, and therefore, that wrong deductions are likely to be drawn when the total ventilation and rate are alone studied. We do not wish to underestimate the importance of observing respiration rate, but we do wish to call attention to the fact that rate has nothing to do, per se, with lung ventilation. Ventilation of the alveolae is carried out entirely, except in such cases of abnormal hyperpnea as that due to pain or emotion, through the chemical stimulation of the respiratory center by the hormone carbon dioxid. Also it might be well to add here, that it has been recently shown by Hasselbalch[3] and others that this action is due merely to the fact that carbon dioxid (in solution) is an acid; any other acid will also stimulate the center. In other words, it is apparently the hydrogen ion concentration of the blood reaching the center that stimulates it. When there is an increase in the production of carbon dioxid, as during muscular work, the carbon dioxid content of the blood reaching the center will increase, and hence the acidity will increase until the center by increasing ventilation has restored the alveolar carbon dioxid tension to its former level. This increase in ventilation may be accomplished by an increase in rate, or depth, or both. What determines the method by which ventilation shall be increased, in other words, whether it shall be rate, depth, or both that are increased, is, in all probability, regulated through nervous channels. For instance, the demand for an increased ventilation having been received by the center chemically, the carrying out of this function is probably controlled by afferent nervous impulses. The character of the breathing must accommodate itself to the mechanical condition in the chest and abdomen. Thus in abdominal distention deep breathing may be impossible, and hence a demand for increased ventilation will have to be met by an increase in rate, or, in the case of an embarrassed heart, a sufficient increase in depth may be impossible and an increase in rate must be resorted to.

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[3] Hasselbalch, K. A.: Neutralitätsregulation und Reizbarkeit des Atemzentrums in ihren Wirkung auf die Köhlensaurespannung des Blutes, Biochem. Ztschr., 1912, xlvi, 403.

We are quite ready to admit that observations on total ventilation and rate alone are not without value. For instance, if after giving a drug the rate was found to remain the same and the total ventilation increased, alveolar ventilation must have increased also. If, however, the rate and total ventilation are both increased, the alveolar ventilation might or might not be increased, and the only way to decide the point would be to determine the dead space and calculate the alveolar ventilation. We also wish to emphasize the necessity of studying the metabolism coincidently with alveolar ventilation, for, even though the alveolar ventilation were determined, it would be impossible to tell whether an increase were due to an increased metabolism or to an increased excitability of the center, unless either the carbon dioxid elimination or the alveolar carbon dioxid tension were determined. It is true that, were an increase in alveolar ventilation found and the carbon dioxid tension unchanged, it might be inferred that the metabolism had been increased. It seems more accurate, however, to determine the metabolism directly as a check on the other findings.

The literature contains a large amount of experimental work on the action of drugs on respiration. This work, with a few exceptions, has been done on animals and by far the greater portion is on the

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4. Loewy, A.: Zur Kentniss der Erregbarkeit des Athemcentrums, Arch. f. d. ges. Physiol., 1890, xlvii, 601.

effect of depressants, rather than stimulants. Loewy[4] in 1890 studied the effect of various drugs, chiefly depressants, on the respiration in human subjects. He determined the total ventilation and the increase in it produced by definite increases in the expired carbon dioxid. Camphor is the only one of the drugs that he studied with which we have worked. His results are plotted in the form of curves in which the ordinates are the inspired carbon dioxid percentage and the abscissae the total ventilation in a given time unit. With two subjects after 0.5 gm. of camphor he obtained in one instance a slight increase (over normal) in total ventilation to a given rise in carbon dioxid tension of the inspired air, but with another subject the results were practically negative.

In 1892 Wood and Cerna[5] studied the effect of a number of drugs on total ventilation and the respiration rate in dogs. After strychnin there was nearly always an increase in total ventilation. The rate sometimes was increased, and sometimes not. The dosage of strychnin used was from 0.00007 to 0.00019 gm. per kilo body weight, and was sufficient, in most of the experiments, to produce toxic symptoms.

A similar increase in total ventilation, and usually in respiration rate also, was noted after atropin. In the case of both these drugs, when both rate and total ventilation were increased, the alveolar ventilation must have increased, but since we do not know the alveolar carbon dioxid there is no means of knowing whether the increase was due to increased metabolism or increased excitability of the respiratory center.

The same may be said of the work of Impens,[6] who found an increase in total ventilation and rate after the administration of caffein in rabbits. The same author, with camphor and strychnin, obtained negative results.

Biberfeld[7] also made some observations on strychnin in rabbits. The respiration rate and total ventilation alone were determined and the results were somewhat variable, in some cases there being an increase in total ventilation, in others not.

Dreser[8] in 1898 carried out some extensive researches on the effect of the various members of the morphin group in rabbits. The animals breathed through Müller valves and the total ventilation was measured with a meter. The oxygen absorption was determined with a modified

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[5] Wood, H. C., and Cerna, D.: The Effect of Drugs and Other Agencies upon the Respiratory Movements, Jour. Physiol., 1892, xiii, 870.

[6] Impens: Les analeptiques de la respiration, Arch. de pharmacodynam, 1899, vi, 149.

[7] Biberfeld: Zur Wirkungsweise des Strychnins auf das Athmungscentrum, Arch. f. d. ges. Physiol., 1904, ciii, 266.

[8] Dreser, H.: Ueber die Wirkung einiger Derivate des Morphins auf die Athmung, Arch. f. d. ges. Physiol., 1898, lxxii, 485.

Regnault apparatus. A decrease in both rate and total ventilation was found with these drugs, most marked in the case of heroin. The alveolar ventilation, therefore, must have been decreased and this presumably was due to a depression of the center, because the change was very marked, but again we cannot be certain that it may not have been due, in part at least, to a decrease in metabolism, for the alveolar carbon dioxid was not determined. The consumption of oxygen after codein and heroin in most of the experiments showed a fall, indicating that metabolism probably did decrease; the carbon dioxid elimination unfortunately was not determined.

Cushny[9] in 1913 studied the effect of caffein and strychnin on the depth and rate of respiration, using a modified body plethysmograph and recording tambour. In the case of both these drugs he found the rate increased and the depth slightly decreased. The reaction to carbon dioxid was also tested, and it was found that there was a greater increase in rate after these drugs than before. Here again, not knowing the alveolar carbon dioxid or alveolar ventilation, we are somewhat at a loss to get the real significance of the results obtained.

The present communication is a preliminary one; it is based on a small number of experiments, but the results seem to be sufficiently striking to make it worth while to report them at the present time.

The methods employed were as follows: The subjects were two normal men, both physicians who voluntarily submitted to the investigation. The experiments were done in the morning, before food was taken (except in the case of Experiment 8, in which a cup of coffee had been taken one hour before experiment was begun) in the recumbent position. The observations on respiration were made with Benedict's so-called Universal Respiration Apparatus.[10] By means of this instrument we measured directly the total ventilation, the carbon dioxid output, the oxygen absorption and the rate of the respiration. The apparatus was run for ten-minute periods. The subjects were always kept at rest in the recumbent position for half an hour before the first period was begun and did not leave the couch until the end of the experiment. One, or in some cases two, normal periods were run, the drug was then given, and two to three subsequent periods run. The systolic blood-pressure was taken frequently by means of the Faught mercury apparatus, the figures in the following protocols representing averages of readings just before, just after and during each period. Frequent pulse-counts were made through a long stethoscope strapped to the cardiac area and arranged so that the subject did not know when his pulse was being counted. The figures in the protocols represent averages of three or more counts.

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[9] Cushny, A. R.: On the Pharmacology of the Respiratory Center, Jour. Pharm. and Exper. Therap., 1913, iv, 363.

[10] Benedict, F. G.: Ein Universalrespirationsapparat, Deutsch. Arch. f. klin. Med., 1912, cvii, 156.

The dead space in the chest was determined for each subject by taking the carbon dioxid tension by the Haldane method and calculating the dead space from this, together with the result of a respiration

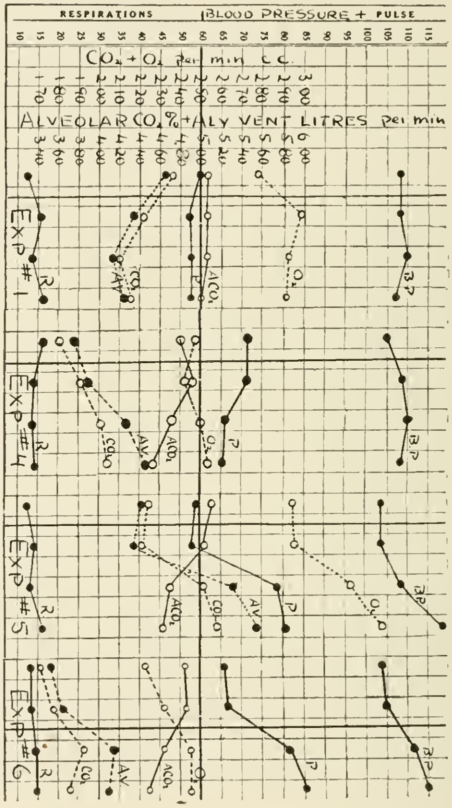

Chart showing diagrammatically Experiment 1 (strychnin), Experiment 4 (caffein), Experiment 5 and Experiment 6 (atropin). The heavy perpendicular line separates periods before, from those after, the administration of the drugs. B. P. = systolic blood-pressure; P = pulse-rate per minute; R = respiration rate per minute; O_2 = oxygen absorbed cubic centimeters; CO_2 = carbon dioxid eliminated, cubic centimeter per minute; ACO_2 = alveolar carbon dioxid per cent.; A. V. = alveolar ventilation liters per minute.

experiment. This was done from several normal periods on the respiration apparatus and Haldane determinations taken on different days, and the results agreeing satisfactorily, the average was used as the value for the dead space in the calculation. The dead space having been found, the alveolar ventilation was obtained by multiplying the dead space by the respiration rate, and subtracting the product from the total ventilation. The percentage of carbon dioxid in the alveolar air has been calculated with sufficient accuracy for our purposes by dividing the carbon dioxid output per minute by the alveolar ventilation per minute. This has been done for each period and the carbon dioxid percentages given in the protocols were obtained in this manner. The alveolar carbon dioxid has been expressed in percentage rather than as actual tension, because, unless there is a marked change in the barometer, changes in percentage run parallel with those in tensions.

When we began the investigation we believed that the size of the dead space remained constant in any individual. We based this belief on Krogh's[11] recent work in which he found no change in the dead space in a given individual under widely varying conditions, such as extreme muscular work or complete rest. For this reason it did not seem important to make frequent direct determinations of the carbon dioxid tension, and we thought that the calculated values would give us the information we desired. In a second series of experiments now in progress in collaboration with H. L. Higgins of the Carnegie Nutrition Laboratory, however, we have obtained evidence that some drugs may alter the size of the dead space by acting as bronchoconstrictors or dilators, as mentioned in the beginning of this paper. Of the four drugs discussed here, the effect has been found with atropin only. The question of changes in the dead space will be discussed when we publish this second series, in which a larger number of drugs have been studied and frequent direct determinations of the carbon dioxid tension made.

We have to report eight experiments at the present time, two with strychnin, two with caffein, two with atropin and two with camphor. One experiment was done with each of the four drugs on each subject. Experiments without drugs have also been done on both subjects, the results of which were perfectly normal. The protocols of the eight drug experiments are given in full, and in addition, Experiments 1, 4, 5 and 6 are shown diagrammatically on a chart.

We wish to thank Drs. O. F. Montgomery and M. N. Smith-Peterson, who not only acted as subjects but also rendered invaluable assistance at the experiments. We also wish to thank Messrs. T. M. Carpenter and H. L. Higgins of the Nutrition Laboratory for much valuable assistance and advice in the use of the respiration apparatus. The experiments in detail were as follows:

REPORT OF EXPERIMENTS

Experiment 1.—*Strychnin.*—Subject O. F. M. After one normal period and during the first third of the second period, strychnin sulphate, 1/15 grain, was given subcutaneously. The three subsequent periods show practically no change in the alveolar carbon dioxid tension and a fall in the alveolar ventilation which is strictly parallel with a fall in the carbon dioxid eliminated per minute; a slight rise in the oxygen per minute also occurs. The systolic blood-pressure shows very little change and the pulse a very slight drop; the respiration rate is practically unaltered.

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[11] Krogh, A., and Lindhard, J.: The Volume of the Dead Space in Breathing, Jour. Physiol., 1913, xlvii, 30.

Experiment 2.—*Strychnin.*—Subject M. N. S-P. In this experiment the dose of strychnin and the method and time of administration were the same as in Experiment 1. We find again a steady fall in the carbon dioxid elimination with a slight rise in the oxygen as in the case of Experiment 1. The alveolar carbon dioxid rises very slightly, and the alveolar ventilation falls somewhat more than in Experiment 1, which is explained by the slight rise in the alveolar carbon dioxid tension. The systolic blood-pressure shows a very slight rise, the pulse a very slight drop, and the respiration rate is unchanged.

Experiment 3.—*Caffein.*—Subject O. F. M. In this experiment caffein sodium salicylate, 5 grains, was given subcutaneously during the first third of the second period and repeated during the first third of the third period. The metabolism shows a definite rise during the second period, and again falls during the third and fourth. The alveolar carbon dioxid tension shows first a rise, then a fall to slightly below the starting-point. Alveolar ventilation shows some rise. Blood-pressure, pulse and respiration are but little affected.

Experiment 4.—*Caffein.*—Subject M. N. S-P. The results are much more striking. Three grains of caffein sodium salicylate were given during the first third of the second period and repeated during the first third of the third period. The metabolism shows a marked rise, the alveolar carbon dioxid tension a marked drop, and the alveolar ventilation a rise which is proportionately greater than the rise in carbon dioxid per minute. Respiration rate is unchanged. Blood-pressure shows a slight rise and the pulse a slight fall.

Experiment 5.—*Atropin.*—Subject O. F. M. After a normal period, atropin, 1/50 grain was given during the second period and repeated during the third period. Dilatation of the pupils, dryness of the throat, dizziness and increase in pulse-rate all occurred, showing that we had given sufficient to produce typical atropin action. The experiment shows a marked rise in metabolism, in blood-pressure, and in pulse-rate, also a definite fall in alveolar carbon dioxid and a rise in alveolar ventilation which is proportionately greater than the rise in carbon dioxid eliminated per minute. Respiration rate shows a very slight rise.

Experiment 6.—*Atropin.*—Subject M. N. S-P. The results of this experiment confirm those of Experiment 5. Here only 1/50 grain of atropin was given between the second and third periods. Pulse and blood-pressure show a marked rise; so also does the metabolism. Alveolar carbon dioxid tension shows a definite drop and alveolar ventilation a rise proportionately greater than the rise in carbon dioxid per minute.

Experiment 7.—*Camphor.*—Subject O. F. M. Camphor, 4 grains, given intramuscularly during the third, and 4½ grains during the first third of the fourth period. Metabolism shows a very slight rise followed by a fall. Alveolar carbon dioxid shows a slight rise, and the alveolar ventilation runs parallel with the carbon dioxid elimination per minute. Blood-pressure and respiration rate show a slight fall; pulse remains nearly level.

Experiment 8.—*Camphor.*—M. N. S.-P. Here only 4 grains of camphor were given between the second and third period. The metabolism remains normal, the alveolar carbon dioxid shows a moderate drop and the alveolar ventilation a corresponding rise; blood-pressure a slight rise, pulse a definite fall, respiration rate unchanged.

EXPERIMENT 1.—Subject O. F. M. Effect of Strychnin *

Time	CO_2 per Min., c.c.	O_2 per Min., c.c.	R. Q.	Total Ventilation per Min., Liters	Alveolar Ventilation per Min., Liters	Alveolar CO_2 Per Cent.	Average Respiration Rate per Min.	Average Pulse-Rate per Min.	Average Systolic Blood-Pressure
10:44 a.m.	236	279	0.85	6.96	4.67	5.06	12.1	60.0	108
11:17 a.m.	221	299	0.74	7.23	4.37	5.06	15.1	55.5	108
11:40 a.m.	210	292	0.72	6.71	4.16	5.05	13.5	56.0	110
12:12 p.m.	213	291	0.73	7.38	4.26	5.00	16.5	55.5	106

* Strychnin sulphate given subcutaneously, 1/15 grain, during first third of second period.

EXPERIMENT 2.—Subject M. N. S-P. Effect of Strychnin *

Time	CO_2 per Min., c.c.	O_2 per Min., c.c.	R. Q.	Total Ventilation per Min., Liters	Alveolar Ventilation per Min., Liters	Alveolar CO_2 Per Cent.	Average Respiration Rate per Min.	Average Pulse-Rate per Min.	Average Systolic Blood-Pressure
10:21 a.m.	192	234	0.82	7.07	3.93	4.89	14.5	72.0	108
10:53 a.m.	189	244	0.77	7.00	3.82	4.95	14.7	71.0	108
11:16 a.m.	180	241	0.75	6.86	3.58	5.03	15.2	67.0	111
11:45 a.m.	184	246	0.75	6.98	3.59	5.12	15.7	69.0	112

* Strychnin sulphate given subcutaneously, 1/15 grain, during second minute of second period.

EXPERIMENT 3.—SUBJECT O. F. M. EFFECT OF CAFFEIN *

Time	CO_2 per Min., c.c.	O_2 per Min., c.c.	R. Q.	Total Ventilation per Min., Liters	Alveolar Ventilation per Min., Liters	Alveolar CO_2 Per Cent.	Average Respiration Rate per Min.	Average Pulse-Rate per Min.	Average Systolic Blood-Pressure
11:00 a.m.	219	275	0.80	6.94	4.63	4.74	12.2	55.0	105
11:41 a.m.	245	316	0.77	7.56	4.86	5.04	14.3	51.5	105
12:17 p.m.	230	300	0.77	7.71	4.95	4.64	14.6	51.5	104
12:44 p.m.	224	309	0.72	7.43	4.82	4.65	13.8	49.5	104

* Caffein sodium salicylate given subcutaneously, 5 grains, during first third of second period and 5 grains during first third of third period.

EXPERIMENT 4.—SUBJECT M. N. S-P. EFFECT OF CAFFEIN *

Time	CO_2 per Min., c.c.	O_2 per Min., c.c.	R. Q.	Total Ventilation per Min., Liters	Alveolar Ventilation per Min., Liters	Alveolar CO_2 Per Cent.	Average Respiration Rate per Min.	Average Pulse-Rate per Min.	Average Systolic Blood-Pressure
10:47 a.m.	180	248	0.73	7.25	3.75	4.80	16.2	72.0	105
11:05 a.m.	192	241	0.80	7.06	3.88	4.95	14.7	72.0	108
11:30 a.m.	201	250	0.80	7.38	4.25	4.72	14.5	66.0	110
11:49 a.m.	205	257	0.80	7.73	4.51	4.54	14.9	65.5	108

* Caffein sodium salicylate given subcutaneously, 3 grains, during first third of second period and 3 grains during first third of third period.

EXPERIMENT 5.—SUBJECT O. F. M. EFFECT OF ATROPIN *

Time	CO_2 per Min., c.c.	O_2 per Min., c.c.	R. Q.	Total Ventilation per Min., Liters	Alveolar Ventilation per Min., Liters	Alveolar CO_2 Per Cent.	Average Respiration Rate per Min.	Average Pulse-Rate per Min.	Average Systolic Blood-Pressure
8:58 a.m.	226	295	0.77	6.80	4.42	5.11	12.6	59.0	104
9:22 a.m.	221	296	0.75	7.16	4.38	5.05	14.7	57.0	104
10:15 a.m.	253	323	0.78	7.87	5.35	4.73	13.3	78.0	108
10:38 a.m.	258	339	0.76	8.74	5.58	4.62	16.7	80.5	118

* Atropin sulphate given subcutaneously, 1/50 grain, during first third of second period and 1/50 grain in middle of third period.

EXPERIMENT 6.—SUBJECT M. N. S-P. EFFECT OF ATROPIN *

Time	CO_2 per Min., c.c.	O_2 per Min., c.c.	R. Q.	Total Ventilation per Min., Liters	Alveolar Ventilation per Min., Liters	Alveolar CO_2 Per Cent.	Average Respiration Rate per Min.	Average Pulse-Rate per Min.	Average Systolic Blood-Pressure
2:27 p.m.	171	222	0.77	658	353	4.85	14.1	66.0	104
2:53 p.m.	178	233	0.77	674	366	4.87	14.2	67.0	105
3:45 p.m.	194	246	0.79	738	416	4.65	14.9	82.0	112
4:15 p.m.	186	246	0.76	735	411	4.52	15.0	87.0	115

* Atropin sulphate given subcutaneously, 1/50 grain, between second and third periods (3:17 p. m.).

EXPERIMENT 7.—Subject O. F. M. Effect of Camphor *

Time	CO_2 per Min., c.c.	O_2 per Min., c.c.	R. Q.	Total Ventilation per Min., Liters	Alveolar Ventilation per Min., Liters	Alveolar CO_2 Per cent.	Average Respiration Rate per Min.	Average Pulse-Rate per Min.	Average Systolic Blood-Pressure
11:01 a.m.	225	285	0.79	6.96	4.48	5.01	13.1	58.0	115
11:28 a.m.	220	278	0.79	7.13	4.09	5.39	16.1	56.0	112
12:09 p.m.	227	304	0.75	6.92	4.35	5.21	13.6	55.0	109
12:34 p.m.	206	277	0.75	6.36	4.04	5.11	12.3	54.5	109

* Camphor (in oil) given intramuscularly, 4 grains, during first third of third period and 4½ grains during first third of fourth period.

EXPERIMENT 8.—Subject M. N. S-P. Effect of Camphor *

Time	CO_2 per Min., c.c.	O_2 per Min., c.c.	R. Q.	Total Ventilation per Min., Liters	Alveolar Ventilation per Min., Liters	Alveolar CO_2 Per Cent.	Average Respiration Rate per Min.	Average Pulse-Rate per Min.	Average Systolic Blood-Pressure
9:37 a. m.	208	240	0.87	7.41	4.08	5.09	15.4	66.5	109
9:56 a. m.	188	239	0.79	7.00	3.74	5.03	15.1	64.0	108
10:50 a. m.	188	237	0.79	7.23	3.90	4.82	15.4	59.0	112
11:35 a. m.	189	238	0.79	7.15	3.95	4.79	14.8	58.0	110

* Camphor (in oil) 4 grains given between second and third periods.

DISCUSSION OF RESULTS

The results obtained with strychnin are clearly negative except possibly for a slight increase in metabolism. The alveolar carbon dioxid in one instance remained constant, and in the other showed a slight rise. The effect on circulation and respiration rate is esssentially negative. It would seem reasonable to conclude that in the case of these two normal individuals strychnin was without recognizable effect on the respiratory center, or the circulation, and also that the general character of respiration is unchanged; it may have slightly increased their metabolism.

Both experiments with caffein show a definite although not very great rise in metabolism. One shows a marked stimulation of the respiratory center as indicated by a fall in the carbon dioxid tension and a rise in the alveolar ventilation; the other shows the same thing, but to a less marked extent.

The experiments with atropin are in perfect agreement and show a definite rise in metabolism, a marked fall in the alveolar carbon dioxid, and a rise in alveolar ventilation. We thought at first that the latter two effects were due to a stimulation of the respiratory center, and they probably are in part, but as we mentioned before, subsequent experiments (to be published later) have indicated that this may also, in part, be due to a change in the size of the dead space.

The results obtained with camphor show no constant effects. In one experiment there was a slight fall in carbon dioxid tension, suggesting that there may have been some stimulation of the center, but in the other experiment there was none. Circulation and respiration rate are essentially unaltered. Metabolism shows a temporary rise in one case, and no effect in the other.

The objection may be raised that these drugs may act differently on sick and on normal individuals. This is highly probable, and we intend to work with pathologic cases later. The effect of the drugs on normal persons, however, should be established first, and the effect on normal men is of even more importance than that on lower animals, which is what has chiefly been studied.

A single observation has been made with atropin in marked Cheyne-Stokes breathing, occurring in a case of cerebral hemorrhage. Assuming that the Cheyne-Stokes breathing may have been due to a decreased excitability of the respiratory center, it seemed reasonable to give a drug which we believed stimulated this center. Enough atropin was given to cause marked increase in pulse-rate; the respiration was recorded by a pneumograph and showed absolutely no change after the administration of atropin. We were unable to obtain the alveolar carbon dioxid tension, as the patient was in coma, and Boothby and

Peabody's[12] pharyngeal tube method for obtaining samples of alveolar air had not been published at the time the case was in the hospital.

In this connection the results of Parkinson and Rowlands[13] studying the effect of strychnin in cases of cardiac decompensation are of interest. These authors found no change in respiratory rate after doses of 1/15 grain of strychnin. They also were unable to observe any alteration in depth, but no means for recording this accurately were employed. In five cases of Cheyne-Stokes breathing absolutely no change in the respiratory curve was found after strychnin.

CONCLUSIONS

We think the following conclusions are justified:

1. Strychnin: No action on the respiratory center or circulation, possibly an increase in metabolism.

2. Caffein: Stimulation of respiratory center, moderate increase in metabolism, no effect on circulation.

3. Atropin: Marked increase in metabolism, in doses large enough to cause the classical action on the circulation. Marked drop in the calculated values for alveolar carbon dioxid and rise in alveolar ventilation. Whether these latter effects are due to stimulation of the respiratory center or to changes in the dead space is not clear at the present writing.

4. Camphor: The results not agreeing, no conclusions are justifiable.

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[12] Boothby, W. M., and Peabody, F. W.: A Comparison of Methods for Obtaining Alveolar Air, The Archives Int. Med., 1914, xiii, 497.

[13] Parkinson, J., and Rowlands, R. A.: Strychnin in Heart Failure, Quart. Jour. Med., 1913, vii, 42.

SOME CONSIDERATIONS ON THE PHYSIOLOGY OF THE OTIC LABYRINTH *

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The philosopher's statement, now more than a century old, that there is nothing in the mind that has not come in through the senses, might be applied to motion in general. Certainly, so far as those movements due to the central nervous system are concerned, we may say that there are extremely few motions that have not had their antecedent afferent impulses. The modern conception of the nervous system regards it as a mechanism in which afferent impulses, arising in the various kinds of peripheral receptors or sensory endings are gathered up into a definite body of sensory impulses, and somewhere in their course, are summed or integrated to a definite orderly motor response.

For example, when a child is learning to write, and is copying a simple figure, afferent impulses are passing in from various peripheral sources, not from the eye alone, but also from the skin, and the muscles which feel and grasp the pencil, and from other sources. The actual movements of the pencil in the fingers are in response to all these impulses, some of which make their way to the cerebrum directly, and others, particularly those which deal with the "unconscious sensation" of the muscles, pursue an indirect path through the cerebellum. But all these afferent impulses, from whatever source or over whatever path, must be associated or added together and harmonized — integrated — within the brain before the fingers can be moved adequately.

The labyrinth of the ear is a circumscribed, definitely located peripheral sense organ, and one task of modern physiology is to inquire into (1) the modes of stimulation of the particular forms of sensory endings which are found in the labyrinth, (2) the effect of the normal afferent impulses from the labyrinth on the normal motor response of the animal, and (3) the particular variations in this motor response occasioned by afferent impulses of unusual violence, e. g., those arising from artificial stimulation or from pathological processes within the ear, or by total lack of such afferent impulses following experimental or pathological loss of the labyrinth.

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* Based on a paper read before the Section on Neurology of the New York Academy of Medicine, Feb. 11, 1913.

Obviously, the detailed consideration of all these matters would transcend the limits of such a paper as the one we now purpose to present, and we will consider, therefore, certain salient points which have impressed themselves on us in the course of a long and rather detailed study of the subject.[1]

Exact experimental work on the semicircular canals began with Flourens in 1828, although other investigators, notably Purkinje, had stimulated the canals in various ways without knowing exactly with what structures they were dealing. Flourens, while experimenting on pigeons, noticed that after removal of one canal, movements of the head occurred in the plane of the destroyed canal, or, as it is sometimes expressed, about an axis perpendicular to the plane of the destroyed canal. Flourens' work has been repeated by many subsequent observers, and the results may now be regarded as firmly established. These specific effects of experimental lesions of the semicircular canals are not due, as some have supposed, to accidental injury to the cerebellum, or to the extension of inflammatory processes to it. Nor does experimental removal of the cerebellum, without injury to the labyrinth or to the vestibular nerves, in our experience on mammals result in exactly the same motor disturbances as elimination of the labyrinth.

In pigeons, removal of one labyrinth is followed in ten to fourteen days by torsion of the head. But removal of the cerebellum in part or wholly, is not followed by such torsion, but by tremors. Removal of the cerebellum and then removal of the labyrinth is followed immediately by the onset of torsion, and the less the amount of cerebellar tissue removed, the greater the delay in the onset of the torsion. This indicates that in pigeons, so far as torsion is concerned, there is something in the cerebellum which resists the onset of torsion, and removal of the cerebellum does not impede its onset. In other words, torsion is essentially an extracerebellar phenomenon. This is quite in keeping with our observations on mammals, with reference to torsion and nystagmus as well.[1]

Removal of the cerebellum in the pigeon, without injury to the labyrinth is, as Lange[2] points out, a matter of some difficulty. Obviously, unless we can get lesions, restricted to the labyrinth without injury to the cerebellum on the one hand, and lesion of the cerebellum alone without involvement of the labyrinth or vestibular tract on the other, we can get no clear or exact picture of the effects of such lesions, and hence no exact basis for a differential diagnosis of pathological lesions of these two mechanisms.

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[1] Wilson and Pike: Philosophical Trans. Royal Soc., London, Series B, 1912, cciii, 127.

[2] Lange: Arch. f. d. ges. Physiol., 1891, l, 615.

Experimental elimination of the labyrinth in the pigeon and many other animals has been accomplished (1) by anatomical removal of the labyrinth or division of the vestibular nerve, and (2) by anesthetizing the labyrinth with cocain. *Stimulation* of the labyrinth has been done by electrical currents, temperature, ice, hot and cold water, hot wire, etc., mechanical pressure, withdrawal of water by crystallin substances applied to the membranous labyrinth, and rotation of the body and particularly of the head, about an axis perpendicular to the plane of some one set of canals. The various forms of stimuli may be classified according to their effects as strong or weak. The stronger stimuli, e. g., closure of the electrical current when the cathode or negative pole is over the labyrinth, hot water, in certain positions of the head in space, strong pressure on the endings of the vestibular nerve, or a fluid current set up either by mechanical pressure on the canal or by placing a crystal of cocain on the horizontal canal in such a position that the *current* is toward the ampulla, cause a movement of the head and eyes in the plane of the canal and *away* from the stimulated side. More moderate stimuli, e. g., the closure of the electrical current when the anode is over the labyrinth, cold water or ice, again with due reference to the position of the head, and withdrawal of water in such a way that the current is away from the ampulla (horizontal canal), cause movement of the head and eyes in the plane of the stimulated canal toward the stimulated side.

The extremely slow currents set up by the slow withdrawal of water from the canals must not be confused with the strongly flowing currents sometimes supposed to be set up in the semicircular canals by change of aspect of the head in space or by irrigation of the external auditory meatus with hot and cold water.

Much discussion has arisen as to whether the effects of unilateral anatomical removal are due to simple absence of one labyrinth, or to irritation of the nerve endings and nerve trunk in the operation wounds. We may safely say that irritation plays but little part in the effects of anatomical removal since (1) the same results are obtained when the labyrinth is anesthetized with cocain, and (2) the results of irritation, if any be present, are the same as those observed after application of the weaker stimuli to the labyrinth, and (3) the results of removal of the second labyrinth after an interval are much less severe than those observed after removal of the first labyrinth. The results are, in the main, due to the unbalanced action of the intact labyrinth.

Without at this time going into a detailed review of the evidence on which the statement rests, we may say that the effects of elimination of the left labyrinth, either by anatomical removal or anesthesia by cocain, are the same as those following electrical stimulation of the labyrinth with the anode over the mastoid fossa, by the application of cold water to the ear, or by rotation to the right. We may, then, consider at this time, the specific results of experimental lesions of the labyrinth, of anesthesia, of electrical and thermal stimulation and of rotation, and afterward consider certain theories of the action of the labyrinth.

In point of historical development, the first most important work goes back to Purkinje whose laws of rotation vertigo, as they are now called, were worked out before Flourens did his experimental removals. Although Purkinje's observations were correct in every essential particular, he did not attribute the results to the semicircular canals, but supposed that in rotation the brain exerted traction on certain cranial nerves because it lagged behind the skull in starting or stopping. We may give these laws of rotation vertigo in detail.

We should distinguish between the period during which rotation actually occurs—the rotation period proper—and the period beginning immediately after the cessation of rotation—known as the postrotatory period. If one stands erect, or sits erect in a chair, and rotates, say to the right, objects may apparently move to the left and one has a sensation of real movement to the right. Shutting the eyes excludes the apparent movement of external objects, but does not affect the sensation of movement of one's own body. If one stands erect and rotates about a vertical axis for some minutes and then stops, surrounding objects still appear to move around and vertigo may be present in the postrotatory period. The direction of the apparent motion of oneself depends on the directions of the preceding real motion and is always opposite in direction. If one bends the head forward 90 degrees and then rotates, the apparent movement during the postrotatory period is about a vertical axis. But if, after again bending the head forward and rotating, the head is raised to an erect position during the post rotatory period, the apparent movement is no longer about a vertical but about a horizontal axis. Objects will seem to rise on one side and fall on the other. The axis about which apparent movement occurs is always the axis of the head about which the preceding real rotation occurred. Hitzig, in his classical paper, gave the first clear and accurate account of the results of electrical stimulation of the labyrinth—galvanic vertigo—and showed that these results fall into line with those of rotation vertigo. When the cathode is placed over the left fossa mastoidea and the anode over the muscles of the neck, the eyes move slowly to the right and quickly back to the left. The subject may get the impression of apparent movement of his own body to the left; the apparent movement of external objects is frequently to the left, but varies with the individual. Closing the eyes abolishes the impression of apparent movement of external objects, but does not abolish the sensation of apparent movement of one's own body. As Hitzig expressed the relation between stimulating electrode and direction of eye movement, the quick movement of the eyes is in the direction of flow of the electrical current, i. e., in the case just cited, toward the cathode.

Hitzig[3] was among the first to stimulate the labyrinth with cold, although he did not know at the time that it was the labyrinth he was stimulating. Bornhardt,[4] a few years later, employed the direct application of heat and cold to the labyrinth as stimulating agents. Goltz[5] referred to the disturbance of equilibrium and the eye movements following incautious irrigation of the external auditory meatus, especially when the tympanic membrane was ruptured, as being common knowledge in his day, and attributed the effects to stimulation of the semicircular canals and the labyrinth generally by the water. From these early beginnings, there has grown up the caloric test as applied in clinical practice of the present day, largely due to the work of Bárány.

Anatomical removal of the labyrinth in mammals, e. g., dogs and monkeys, is accompanied by certain characteristic phenomena. Certain of these are acute, lasting but a few days, while others persist as long as the animal lives. If one labyrinth only is destroyed, there is a slow movement of the eyes toward the injured side, and a quick return jerk to the median position. This nystagmus persists but a few days, and then wholly ceases, the eyes being then normal in position and capability for movement. In lower forms, such as the dog-fish[6] and turtle,[7] the eyes are permanently deviated toward the injured side, and there is no nystagmus.

Although the eyes in mammals appear to come back to the normal position in a few days, we have repeatedly observed that if the animal be placed in an abnormal posture, e. g., on its back, the symmetrical position of the eyes is altered and there will be observed a marked ventral deviation of the eye on the side of the lesion. This persists for weeks. The animal's head is permanently turned toward the injured side, and, in the early days of the postoperative period, the animal falls or rolls to the injured side. Later the animal becomes able to walk without falling, but turns toward the injured side so that it goes in a circle. In a few days more, it is able to walk a straight line, even when its eyes are covered. The transitory falling over of the animal to the side of the lesion and the persistent deviation of the head is, we believe, due to the over activity of the intact labyrinth, whereby the muscles, chiefly the extensors on the intact side, are acting more effectively.

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[3] Hitzig: Arch. f. Anat. u. Physiol., 1871, p. 771.

[4] Bornhardt: Arch. f. d. ges. Physiol., 1876, xii, 471.

[5] Goltz: Arch. f. d. ges. Physiol., 1870, iii, 192.

[6] Jour. Physiol., 1894, xv, 311; 1895, xvii, 192.

[7] Wilson and Pike: Proc. Soc. Exper. Biol. and Med., 1913, xi, 52.

If both labyrinths are removed at one time, there is nystagmus as before, but it is irregular in character and does not persist as long as after unilateral removal. There is no torsion of the head to one side or the other, or at most, a comparatively slight inclination. The animal refuses to stand, but lies with head and feet outstretched and paws clutching the floor. If the head is raised voluntarily, there are coarse swaying movements reminding one of the swaying of a reptilian head. Food is not accurately grasped with the jaws. In attempting to take food, the nose is thrust to one side, above or below the desired object, only rarely touching the object squarely. A turtle deprived of both labyrinths may die of starvation because of its inability to seize food. In the dog and cat, the swaying movements cease in two weeks or less, and the accuracy of grasp returns. Gradually, also, the cat or dog learns to walk, falling to one side or the other in the first attempts, and turning now to one side, now to the other in its efforts to cross a room. Finally, all these symptoms disappear, and it would be difficult for one to detect any motor disturbance in a dog with eyes unbandaged in walking across grass or a level floor, or in taking food.

The eye movements are of particular interest in connection with stimulation or removal of the labyrinth, and we may inquire into them in detail.

In all animals so far experimented on there is a slow movement of the eyes toward the injured labyrinth or toward the labyrinth to which the weaker stimulus is applied. In rotation, the eyes lag behind, going slowly to the side opposite to that toward which the body is rotated. In the postrotatory period the slow movement of the eyes changes and acquires the same direction as the preceding real rotation. The typical oscillatory movement of the eyes, alternately slow in one direction and quick in the opposite direction, is seen best in mammals. So far as our observation or knowledge goes, this is a characteristic type of nystagmus not seen in experimental lesions of the cerebellum or of any other portion of the central nervous system so long as the vestibular tract is intact. The slow deviation alone is seen in certain of the lower forms. We have found that the slow deviation alone is seen in decerebrated or partially anesthetized animals. Nystagmus in its characteristic form occurs only in those animals which have recovered to a considerable extent from the anesthetic, or in which the optic thalamus with the corpora striata and a considerable portion of the cerebral hemispheres remained. Some part of the mechanism which produces nystagmus must, therefore, be anterior to the corpora quadrigemina. The exact paths concerned will be reported on later.

Bartels, basing his views partly on the afferent nerve endings recently described in the eye muscles by Sherrington and his pupils, and partly on rotation experiments on infants, concludes that the quick return jerk of the eyes in labyrinthine nystagmus is a reflex dependent on some mechanism, probably cortical, but certainly cerebral. Bartels is not inclined to accept Bárány's view of a hypothetical *Blickcentrum* as the cause of nystagmus. Bartels found that in the first seven days of post partum life, rotation nystagmus was not obtainable. Later, it is obtainable when the child is awake, but not during sleep. Prematurely-born infants do not exhibit rotation nystagmus for some time after birth. An infant born at the end of seven months, uterine life does not show rotation nystagmus in the early postpartum period, although anatomically, the semicircular canals are fairly well developed at the end of three months. The time of appearance of rotation nystagmus apparently depends more on the period elapsing after birth than on the absolute length of gestation.

We agree in the main with Bartels' idea of the mechanism of labyrinthine nystagmus, and have published additional evidence of their essential correctness and extended them considerably.

Clinicians and laboratory workers alike have been impressed with the quick phase of nystagmus, and the direction of nystagmus is usually given as the direction of the quick movement. In so doing, all have fixed attention on the mechanism which brings about the return of the eye to the normal position, and not on the lesion which causes the eye to deviate from its normal position. But the cause of the deviation from the normal position is the very thing which the clinician is most anxious to discover. If the experimenter has anything definite to say to the clinician, it is this one thing, that the cause of the slow deviation in labyrinthine nystagmus lies in the labyrinth or in the vestibular tract, and that the slow component of nystagmus is the one which it is most important to observe. The cause of the ocular deviation from the normal position may lie in other parts of the central nervous system than the labyrinth or the vestibular tract, and each may bring about the deviation in a particular way. The manner of return to normal is more likely to be uniform. Involvement of the labyrinth or the vestibular tract always, in experimental procedures at least, produces a relatively slow, wide deviation, in which both eyes move together, and we may repeat that labyrinthine nystagmus is characterized by these alternately slow and rapid movements. As at present used, nystagmus is an indefinite term, covering various types of ocular movements arising from lesions in diverse parts of the nervous system, and a more careful distinction of the different types of eye movement is greatly to be desired.

After the subsidence of the symptoms of the acute stage, certain other permanent defects are observable.

If but one labyrinth has been removed, there is a permanent torsion of the head to the injured side, and there may be a leaning, particularly of the anterior part of the body, to this side. This torsion of the head is much more marked in animals than in the human subject. In the latter, the torsion may be negligible. If both labyrinths have been removed, either at one sitting or at intervals, there is no marked torsion of the head or inclination of the body to one side. With eyes bandaged, the animal is unable to walk in a straight line, but goes now to one side, now to the other, or turns helplessly in a small circle. Ascending stairs offers no particular difficulty, but descending stairs usually results in a headlong fall. Jumping down from even a moderate height results in falling in a heap on the floor. An animal with one labyrinth intact walks in a straight line when blindfolded, ascends or descends stairs and jumps up or down without difficulty. In neither animal is there any loss of the sense of position of the muscles, so far as can be made out. The drop reflex — the thrusting out of the hind feet and legs when the animal is held vertically, head up, and rather rapidly lowered with the hands — is greatly depressed or wholly absent on the side of labyrinthine injury. In no case can any confusion concerning the relation of the parts of the body to each other, or to the body as a whole, be made out. The animal's mistakes and failures concern his relation to external objects or his orientation in space.

Such briefly and in general are the results of labyrinthine removal. Various theories have been put forward to explain these defects. We may mention certain of them. The first clear conception is due to Goltz, who pointed out that, in the labyrinth and its central connections we had, first, a peripheral sense organ in the semicircular canals and the other portions of the otic labyrinth; second, a centripetal path from this end organ to the central mechanism, and finally certain centrifugal paths from the central mechanism to the muscles, through which the motor response is brought about. The function of the labyrinth is, therefore, a reflex one.

Goltz, however, supposed that the end organs were stimulated by changes in hydrostatic pressure in the labyrinth as the animal moved its head about in space. Although certain remnants of this hydrostatic theory still survive and are likely to survive for some time to come, it has been largely superseded by the kinetic theory of Mach, Breuer and Brown, particularly with reference to the semicircular canals. As Brown expressed it, on the basis of the hydrostatic theory, and static theories in general, the labyrinth is regarded as an organ which gives information of the aspect of the head in space. In the kinetic theory, the labyrinth, and especially the semicircular canals, are regarded as an organ which gives information of the change of aspect of the head in space. The distinction is important, for, in response to rapid changes of aspect of the head in space, prompt and rapid motor reactions are necessary to preserve equilibrium or to break a fall. There is no other sense organ in the body which will give this information quite so efficiently as the labyrinth, and hence, there is always a certain amount of permanent deficiency of motor response after total loss of the labyrinths.

Breuer's original idea was that because of its inertia, the fluid in the canals lagged behind when the head was moved in any plane, and the sensory endings in the labyrinth were stimulated by fluid currents. Mach objected to the currents, arguing that from the point of view of experimental physics, currents were impossible in such fine tubes as the semicircular canals; the effective stimulus must be, therefore, a change in pressure in particular regions of the labyrinth, since the fluid would lag behind sufficiently to cause a change in pressure. Breuer and Brown accepted Mach's modifications of the original theory, and it is generally held to-day that changes in fluid pressure, not fluid current, are the effective or adequate stimuli for the sensory endings in the labyrinth. So far as we can see, it is not necessary to assume the existence of actual currents for the explanation of any known response to stimulation of the labyrinth.

Brown showed that the six canals might be divided into three pairs, each pair lying in a plane at right angles to the planes of the other two pairs. Lee showed that each canal in a given pair had two functions, a principal and a subordinate function; and that the subordinate function of each canal of a pair reinforces the principal function of the other canal. As an illustration of this point we have Breuer's statement that, in the horizontal canals, change of pressure from canal to ampulla is a stronger stimulus than change of pressure from ampulla to canal.

Time does not permit a consideration of some of the *"Streitfragen"* of labyrinthine physiology. It may be shown that, under certain conditions, the labyrinth exerts an influence on the state of contraction of certain of the muscles of the limbs. How great this influence is under normal conditions is a matter of conjecture. Again, Stefani's idea that the labyrinth exerted its effect mainly over paths passing through the cerebellum is open to question in part at least. Certainly, typical labyrinthine nystagmus may be induced in a dog after complete ablation of the cerebellum.

Our results are more in line with Luciani's. We believe that while the labyrinthine reaction is primarily a direct reflex effect through the extracerebellar structures, yet the connection with the cerebellum is such that in higher forms at least, the labyrinthine reactions are influenced by the cerebellum.

Finally, the path which labyrinthine impulses follow in going to the cerebrum, or in what part of the cerebral cortex the end stations lie, will be dealt with in a subsequent paper. That labyrinthine impulses do reach the cerebrum is scarcely to be doubted.

INDEX TO VOLUME XIV

PAGE
Abderhalden reaction, a point to be considered in the use of; E. L. Ross and H. D. Singer 552
Altitude and lymphocyte increase; M. E. Staines, T. L. James and C. Rosenberg 376
Andrews, Vernon L.: The potassium content of cerebrospinal fluid in various diseases 536
Anemia, aplastic, study of a case of; J. H. Musser, Jr 275
Archibald, R. A.: The production, action and therapeutic effects of leukocytic extracts 120
Artery, brachial, pulse-flow in. IV. Reflections of the primary wave in dicrotic and monocrotic pulse-forms; A. W. Hewlett 609
Ash, J. Earle: The blood in inanition 8

Baetjer, F. H.: Pulmonary physical signs and Roentgen-ray findings in healthy adults 757
Bishop, F. Warner: Elliptical human erythrocytes 388
Blatherwick, N. R.: The specific rôle of foods in relation to the composition of the urine 409
Blood-corpuscles, red, color-index of; E. Meyer and E. E. Butterfield 94
Blood in health and in disease, the total and non-protein nitrogen and the urea of, as estimated by Folin's methods; W. Tileston and C. W. Comfort 620
Blood-platelets and tuberculosis; G. B. Webb, G. B. Gilbert and L. C. Havens 743
Blood-pressure in medicine and surgery, book review; by E. H. Goodman 462
Blood-sugar, a comparison of Bang's micromethod with Bertrand's method for determining; R. Fitz 133
Blumer, George: The normal peculiarities of the heart-sounds in the region of the sternum 605
Book Reviews:
    Blood-pressure in medicine and surgery; by E. H. Goodman 462
    Diseases of the stomach, including dietetic and medicinal treatment; by G. R. Lockwood 143
    A practical treatise on medical diagnosis for students and physicians; by J. H. Musser 461
    Modern medicine. Its theory and practice in original contributions by American and foreign authors; by Sir. W. Osler 608
    The fundamental basis of nutrition; by G. Lusk 143
Botulism: a report of food-poisoning apparently due to eating of canned string beans, with pathological report of a fatal case; R. L. Wilbur and W. Ophüls 589
Bridgman, E. W.: A normal, presystolic sound 475
Bronfenbrenner, J.: The complement-deviation test with Besredka's tuberculin and the occurrence of tuberculosis among syphilitics as diagnosed by this test 786
Butterfield, E. E.: The color-index of the red blood-corpuscles 94

PAGE
Caffein, clinical studies in; L. Taylor 769
Capps, Joseph A.: An epidemic of streptococcus sore throat 650
Carbohydrate tolerance tests, the non-specificity of; J. C. Friedman and S. Strouse 531
Cardiac and renal disease, studies of acidosis and dyspnea in; F. W. Peabody 236
Cerebrospinal fluid, the potassium content of, in various diseases; J. Rosenbloom and V. L. Andrews 536
Christian, Henry A.: The effect of theobromin sodium salicylate in acute experimental nephritis, as measured by the excretion of phenolsulphonephthalein 827
Claypole, Edith J.: Human streptotrichosis and its differentiation from tuberculosis 104
Claypole, Edith J.: Methods of prophylactic immunization against typhoid fever 671
Claypole, Edith J.: Specific hyperleukocytosis 662
Cohoe, Benson A.: Myotonia congenita — Thomsen's disease 263
Cole, Rufus: Pneumococcus infection and lobar pneumonia 56
Coleman, Warren: The influence of the high-calory diet on the respiratory exchanges in typhoid fever 168
Comfort, C. W.: The total non-protein nitrogen and the urea of the blood in health and in disease as estimated by Folin's methods 620
Cretin, nitrogen and sulphur metabolism in; I. Greenwald 374

Davis, David John: An acid-fast streptothrix (Nocardia) 1
Davis, David J.: An epidemic of streptococcus sore throat 650
Dementia praecox, note on the incidence of status lymphaticus in; H. Emerson 881
Diabetes insipidus, a case of; R. Fitz 706
Diagnosis, medical, a practical treatise on, for students and physicians, by J. H. Musser; book review 461
Diller, Theodore: Family periodic paralysis, a typical case, with metabolism study 869
DuBois, Eugene F.: The influence of the high-calory diet on the respiratory exchanges in typhoid fever 168

Edsall, D. L.: The effect of strychnin, caffein, atropin and camphor on the respiration and respiratory metabolism in normal human subjects 897
Emerson, Haven: The incidence of status lymphaticus in dementia praecox 881
Epileptics, the pituitary gland in; J. F. Munson and A. L. Shaw 393
Erythrocytes, elliptical human; F. W. Bishop 388

Fitz, R.: Comparison of Bang's micromethod for determining blood sugar with Bertrand's method 133
Fitz, R.: Diabetes insipidus 706
Friedman, J. C.: Experimental pyloric stenosis 722
Friedman, Joseph C.: The non-specificity of carbohydrate tolerance tests .. 531
Frothingham, Channing, Jr.: The relation between the phenolsulphonephthalein excretion in the urine and the non-protein nitrogen content of the blood in human cases 541

Garrison, P. E.: Introduction to the second progress report of the Thompson-McFadden pellagra commission 289
Garrison, P. E.: The relation of methods of disposal of sewage to the spread of pellagra 453
Garrison, P. E.: The relation of pellagra to use of certain foods and to location of domiciles 293

PAGE

Gay, Frederick P.: Methods of prophylactic immunization against typhoid fever 671
Gay, Frederick P.: Specific hyperleukocytosis 662
Gilbert, G. Burton: Artificial pneumothorax 883
Gilbert, George Burton: Blood-platelets and tuberculosis 743
Glycosuria, postanesthetic, as influenced by diet, body temperature and purity of the ether; E. L. Ross and P. B. Hawk 779
Goodman, Edward H.: Blood-pressure in medicine and surgery; review of 462
Greenwald, Isidor: Nitrogen and sulphur metabolism in a cretin 374

Hamburger, W. W.: Experimental pyloric stenosis 722
Hamman, Louis: Pulmonary physical signs and Roentgen-ray findings in healthy adults 757
Havens, Leon C.: Artificial pneumothorax 883
Havens. Leon C.: Blood-platelets and tuberculosis 743
Hawk, P. B.: Postanesthetic glycosuria as influenced by diet, body temperature and purity of the ether 779
Heart-sounds in the region of the sternum, a note on the normal peculiarities of; G. Blumer 605
Hemorrhage, the pathologic physiology of the circulation during; C. J. Wiggers 33
Hepatic functional tests, two (galactose and phenoltetrachlorphthalein), a clinical study of; W. R. Sisson 804
Hewlett, Albion Walter: The pulse-flow in the brachial artery 609
Hexamethylenamin as a urinary antiseptic, a clinical and bacteriological study of; L. Henry Levy and A. Strauss 730
Hyperleukocytosis, specific; studies in typhoid immunization, IV; F. P. Gay and E. J. Claypole 662

Inanition, the blood in; J. E. Ash 8

James, T. L.: Artificial pneumothorax 883
James, T. L.: Lymphocyte increase and altitude 376

Kessel, Leo: Advanced pulmonary tuberculosis treated by artificial pneumothorax 210
King, John H.: The pathology of the spleen 145

Lamb, Albert R.: Periarteritis nodosa 481
Leukocytic extracts, a preliminary report of the production, action and therapeutic effects of; R. A. Archibald and G. Moore 120
Levy, Louis Henry: Hexamethylenamin as a urinary antiseptic 730
Lockwood, George Roe: Diseases of the stomach, including dietetic and medicinal treatment, review of 143
Lusk, Graham: The fundamental basis of nutrition, review of 143

MacNeal, W. J.: Introduction to the second progress report of the Thompson-McFadden pellagra commission 289
MacNeal, W. J.: The relation of methods of disposal of sewage to the spread of pellagra 453
MacNeal, W. J.: The relation of pellagra to use of certain foods and to location of domiciles 293
Major, Ralph H.: The glycyl-tryptophan reaction in meningitis 383
McCrae, Thomas, and Osler, William: Modern medicine, review of 608
Means, J. H.: The effect of strychnin, caffein, atropin and camphor on the respiration and respiratory metabolism in normal human subjects 897

PAGE
Medicine, modern, by W. Osler and T. McCrae; review of................ 608
Meningitis, the glycyl-tryptophan reaction in; R. H. Major and E. Nobel.. 383
Meyer, Erich: The color-index of the red blood-corpuscles............... 94
Minot, George R.: The blood-pressure in pneumonia...................... 48
Moore, Gertrude: The production, action and therapeutic effects of leukocytic extracts 120
Mosenthal, Herman O.: Nitrogen metabolism and the significance of the non-protein nitrogen of the blood in experimental uranium nephritis... 844
Munson, J. F.: The pituitary gland in epileptics 393
Musser, John H., Jr.: Aplastic anemia.................................. 275
Musser, John H.: A practical treatise on medical diagnosis for students and physicians, review of.. 461
Myotonia congenita — Thomsen's disease, clinical and metabolism studies in a case of; J. Rosenbloom and B. A. Cohoe.......................... 263

Nephritis, acute experimental: the effect of theobromin sodium salicylate in, as measured by the excretion of phenolsulphonephthalein; H. A. Christian 827
Nephritis, experimental uranium, nitrogen metabolism and the significance of the non-protein nitrogen of the blood in; H. O. Mosenthal.......... 844
Nephritis of children, latent hypertrophy of the heart in the; H. Wessler... 517
Newburgh, L. H.: The blood-pressure in pneumonia...................... 48
Nobel, Edmund: The glycyl-tryptophan reaction in meningitis............ 383
Nutrition, the fundamental basis of, by G. Lusk; review of............... 143

Ophüls, William: Botulism: a report of food-poisoning apparently due to eating of canned string beans.. 589
Osler, William, and McCrae, Thomas: Modern medicine, review of....... 608
Otic labyrinth, some consideration on the physiology of; J. G. Wilson and F. H. Pike 911

Paralysis, family periodic, report of a typical case, with metabolism study; T. Diller and J. Rosenbloom.. 869
Peabody, Francis W.: Acidosis and dyspnea in renal and cardiac disease.. 236
Pellagra, a statistical study of the relation of, to use of certain foods and to location of domiciles in six selected industrial communities; J. F. Siler, P. E. Garrison and W. J. MacNeal............................ 293
Pellagra commission, Thompson-McFadden, introduction to the second progress report of; J. F. Siler, P. E. Garrison and W. J. MacNeal..... 289
Pellagra, the relation of methods of disposal of sewage to the spread of; J. F. Siler, P. E. Garrison and W. J. MacNeal......................... 453
Periarteritis nodosa — a clinical and pathological review of the disease, with a report of two cases; A. R. Lamb............................. 481
Phenolsulphonephthalein excretion in the urine and the non-protein content of the blood in human cases, relation between; C. Frothingham, Jr., and W. G. Smillie.. 541
Pike, F. H.: Some consideration on the physiology of the otic labyrinth.. 911
Pneumonia, lobar, and pneumococcus infection; R. Cole................... 56
Pneumonia, the blood-pressure in; L. H. Newburgh and G. R. Minot...... 48
Pneumothorax, artificial, with report of gas analyses and experiments to determine the use of air or nitrogen; G. B. Webb, G. B. Gilbert, T. L. James and L. C. Havens....................................... 883
Presystolic sound, normal, notes on; E. W. Bridgman..................... 475
Pulmonary physical signs and Roentgen-ray findings in healthy adults; L. Hamman and F. H. Baetjer 757

PAGE
Renal and cardiac disease, studies of acidosis and dyspnea in; F. W. Peabody 236
Respiration and respiratory metabolism in normal human subjects, the effect of strychnin, caffein, atropin and camphor on; D. L. Edsall and J. H. Means 897
Rosenberg, Carolyn: Lymphocyte increase and altitude........ 376
Rosenbloom, Jacob: Family periodic paralysis, a typical case, with metabolism study 869
Rosenbloom, Jacob: Myotonia congenita — Thomsen's disease........ 263
Rosenbloom, Jacob: The potassium content of cerebrospinal fluid in various diseases 536
Ross, E. L.: Postanesthetic glycosuria as influenced by diet, body temperature and purity of the ether........ 779
Ross, Ellison L.: The Abderhalden reaction........ 552

Shaw, Arthur L.: The pituitary gland in epileptics........ 393
Siler, J. F.: Introduction to the second progress report of the Thompson-McFadden pellagra commission........ 289
Siler, J. F.: The relation of methods of disposal of sewage to the spread of pellagra 453
Siler, J. F.: The relation of pellagra to use of certain foods and to location of domiciles 293
Singer, H. Douglas: The Abderhalden reaction........ 552
Sisson, Warren R.: A clinical study of two hepatic functional tests (galactose and phenoltetrachlorphthalein)........ 804
Smillie, Wilson G.: The relation between the phenolsulphonephthalein excretion in the urine and the non-protein nitrogen content of the blood in human cases 541
Spleen, studies in the pathology of; J. H. King........ 145
Staines, Minnie E.: Lymphocyte increase and altitude........ 376
Stenosis, experimental pyloric; W. W. Hamburger and J. C. Friedman.... 722
Stomach, diseases of, including dietetic and medicinal treatment, by G. R. Lockwood; review of 143
Stomach, contributions to the experimental pathology of; I. Experimental pyloric stenosis; W. W. Hamburger and J. C. Friedman........ 722
Strauss, Abram: Hexamethylenamin as a urinary antiseptic........ 730
Streptococcus sore throat in Jacksonville, Ill., an epidemic of, which was traced to the milk of cows affected with streptococcus mastitis; J. A. Capps and D. J. Davis........ 650
Streptothrix, an acid-fast (Nocardia); D. J. Davis........ 1
Streptotrichosis, human, and its differentiation from tuberculosis; E. J. Claypole 104
Strouse, Solomon: The non-specificity of carbohydrate tolerance tests.... 531
Syphilitics, the occurrence of tuberculosis among, as diagnosed by the complement-deviation test with Besredka's tuberculin; J. Bronfenbrenner.. 786

Taschman, Max: Advanced pulmonary tuberculosis treated by artificial pneumothorax 210
Taylor, Lester: Clinical studies in caffein........ 769
Tileston, Wilder: The total non-protein nitrogen and the urea of the blood in health and disease, as estimated by Folin's methods 620
Tuberculosis, advanced pulmonary, treated by artificial pneumothorax, a study of twenty-nine cases of; L. Kessel and M. Taschman........ 210
Tuberculosis among syphilitics as diagnosed by the complement-deviation test with Besredka's tuberculin; J. Bronfenbrenner 786

INDEX TO VOLUME XIV

PAGE

Tuberculosis and blood-platelets; G. B. Webb, G. B. Gilbert and L. C. Havens ... 743
Typhoid fever, an experimental study of methods of prophylactic immunization against, studies in typhoid immunization, V; F. P. Gay and E. J. Claypole ... 671
Typhoid immunization, studies in, IV, specific hyperleukocytosis; F. P. Gay and E. J. Claypole ... 662
Typhoid fever, the influence of the high-calory diet on the respiratory exchanges in; W. Coleman and E. F. DuBois ... 168

Urine, the specific rôle of foods in relation to the composition of; N. R. Blatherwick ... 409

Walker, I. Chandler: The specificity of cholesterin with syphilitic serums and of cholesterin-reinforced heart antigen in the Wassermann reaction 563
Wassermann reaction, the specificity of cholesterin with syphilitic serums and of cholesterin-reinforced heart antigen in; I. C. Walker ... 563
Webb, Gerald B.: Artificial pneumothorax ... 883
Webb, Gerald B.: Blood-platelets and tuberculosis ... 743
Wessler, H.: Latent hypertrophy of the heart in the nephritis of children.. 517
Wiggers, Carl J.: The pathologic physiology of the circulation during hemorrhage ... 33
Wilbur, Ray Lyman: Botulism: a report of food-poisoning apparently due to eating of canned string beans ... 589
Wilson, J. Gordon: Some consideration on the physiology of the otic labyrinth ... 911

R
11
A87
v.12
cop.2
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medicine

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